

Reform for Soviet academy

The Soviet Academy of Sciences seems to have settled down for the next five years, with all the dangers that may bring that urgent reforms will be postponed.

THE Soviet Academy of Sciences is the Soviet Union in microcosm, where not much changes. Our Moscow correspondent (see page 101) has a familiar tale to tell: people know that there is a great deal wrong with the academy, many of the causes have been accurately identified, as have been some of the potential remedies, yet the process of reform seems always to be too difficult to be attempted yet. Maybe tomorrow will bring a more propitious time?

Yet, perhaps after the Bolshoi Ballet, the academy is the most distinguished of Soviet institutions. Proposals for reform abound, the promise of reform is in the air, but nothing much happens. Indeed, Dr Guri Marchuk, re-elected president last month, plainly considers that gradualism will suffice to get from here to a better there — last month he was looking forward to “the steady enhancement of the autonomy of our institutions . . . the gradual erosion of the rigid administrative framework”. Will that suffice?

Much, of course, has changed during Marchuk's spell in office, which coincides almost exactly with that of Gorbachev. *Glasnost* has arrived and is practised, if sometimes shyly, often-unruly democracy touches even the election of laboratory directors and people's travel is restricted in ways with which others are familiar — shortages of time and cash, for example. Fewer decisions about the direction of research are made by grand old men, more by working scientists at the bench. All that is to the good, but the contradictions at the heart of the academy's constitution remain virtually unresolved.

Defects

Stalinist academies everywhere share several defects. They simulate Western-style academies, but their distinction is compromised by the appointment of people for other than academic reasons. (The Soviet academy is less corrupted than others in this respect.) They are also meant to manage research (including researchers) and much of the process of technical innovation, but the task is physically and conceptually impossible. For one thing, especially because researchers must be housed as well as directed, the task is huge. For another, by their near-complete divorce from productive enterprises and their markets, they cannot judge which innovations make economic sense and which do not — and are thus often unfairly blamed by governments for their failure to

deliver what they have implicitly promised. But their near-divorce from higher education means that their capability for research is diminished and their potential influence in society truncated.

In ideal circumstances, these difficulties might be surmountable. In the Soviet Union, they are not. The Party apparatus, however much its influence has declined, still adds an extra dimension of bureaucracy to every decision. Then people, their sense of deprivation sharpened by *glasnost*, are underpaid and badly fed. The immobility occasioned by the housing problem is crippling. The Soviet academy's strength is its army of able and talented researchers drawn to the practice of science by an idealism familiar to an earlier age. The cruelty is that even the ablest people are often denied by circumstances the chance to function as they might. So what, as Lenin asked, is to be done?

Dangers

Gradualism, however convenient, is unlikely to succeed. Indeed, there are even dangers in that course, which risks the disaffection (even emigration) of the academy's most impatient souls. The most serious danger is that the academy's army of people, freed at last from the principle that all direction comes from above, but not yet liberated from the daily sense of personal deprivation, will embrace instead the belief that research is a kind of state institution, entitled to a living, if only a meagre one. The men and women able and willing to take research projects by the scruff of the neck and make them hum along demonstrate, by their exceptional character, that Russia has too few of them. After 70 years, that is no surprise.

What the Soviet academy most needs, though, is a plan for the more distant future. What will that be? The academy should become an academy in the Western sense, and should be ready to give the Soviet government advice (which it does at present, but mostly in private). As things are in the Soviet system, it is also the natural source of competitive funds for research, but it will succeed in that role only if it can strike and then keep a fair balance between the universities and its own basic research institutes. The need is accepted, but the academy will have to work hard on the consciences of its members and their fellow committee-members to avoid the mindless application of the Peter principle — “To him who hath shall be given” and all that entails.



Where should research stand? There is only one solution: the academy should plan now to get out of the management of research at some foreseeable and not-too-distant point in the future. That does not imply that the academy's research is second rate. On the contrary, as the world knows, much of what the academy's institutes do is excellent by any standards. The difficulty is simply that there is an unavoidable conflict between the sponsorship and the management of research. Past errors may be concealed by throwing money at them. Laboratories long since asleep may survive if they have a powerful protector. But the excellent laboratories are cut down to size by envy.

The remedy? Many institutes should be transferred to universities. Other groups of institutes might become universities in their own right. Applied research laboratories, invariably head and shoulders above the research institutes of the production ministries, should be used as means of enlivening those ministries, which are nothing but still-nationalized industries. But that sounds like a revolution, the academy (and others) will say. Why not? It would not be the first time that the Soviet Union had chosen that path in search of a solution for obdurate problems.

Delay

The people problem is more difficult. Housing (or the lack of it), the immobility that results and the general impoverishment of people are the immediate problems. But there is a more serious problem on the horizon: the Soviet research establishment is larger by a factor of some small integer, perhaps five, than even the Soviet Union needs (or will be able to afford when salaries are minimally improved). This does not imply that there will be no jobs for trained technical people to do, but they will be different kinds of jobs. The academy and the institutions that will succeed it in the management of research should plan now for this impending upheaval, devising the incentives that will make it possible and humane. All that should keep Marchuk busy in his second term.

The parallel with the task facing Mr Mikhail Gorbachev for the past five years is all too close, and even discouraging. Gorbachev's ambition to reform the Soviet economic system, for example, looks like running into the sand. For at least the past three years, energetic people have been pleading for economic change. It is not a matter of ideology (Marxism), but of good sense. If bread is cheaper than animal feed, people will feed their pigs on it. If all subway rides cost only 5 kopeks, people may choose to ride when they might walk, or so as to keep warm. If housing is dirt cheap, but too often in an employer's gift, people will be prevented from changing jobs. But now the promises of change have been postponed again, while tax rules applied to cooperatives (the Soviet Union's small businesses) are graduated in such a way as to keep them small and fragmented. Good intentions and logic are defeated, it seems, by inertia and the size of the system. The academy deserves better than that. □

Business as usual

British MPs are confusing embryo research and abortion. Why not cut the gordian knot?

A COMFORTABLE majority of British MPs has decided to allow carefully regulated research on human embryos up to 14 days after fertilization, and to allow pregnant women abortions up to 24 weeks after conception, prompting Cardinal Basil Hume, head of the Catholic church in England and Wales, to remark that Britain is no longer a Christian society. But the controversy generated by these decisions disguises the fact that nothing much will change once the Human Fertilisation and Embryology Bill passes through its committee stage and becomes law.

In the five years that it has taken the British government to find the nerve to introduce legislation based on the Warnock report, researchers themselves set up the Voluntary Licensing Authority to regulate embryo research (see, for example, *Nature* 344, 690; 19 April 1990). Their assumption that legislation would eventually materialize must often have seemed unwarranted — and in the event, the law on embryo research has turned out to be merely a rubber-stamp procedure for the researchers' own guidelines.

The abortion question, awkwardly tacked on to the embryo bill by a familiar collection of hard-line opponents to the practice, was settled by a series of votes, MPs taking their pick from a selection of time-limits on offer. The medical establishment, in the shape of recommendations by the Royal College of Obstetricians and Gynaecologists, won the day with a limit of 24 weeks. MPs' confusion over the issue was reflected in the voting figures: 9 who voted for the 'liberal' abortion option of 24 weeks voted against embryo research, which is thoroughly illogical. And 64 of the 364 who voted for a more conservative 22-week limit had previously voted in favour of embryo research, perhaps reflecting the ethically more complex status of the *in vitro* embryo compared with the *in situ* fetus. This was, in Cardinal Hume's words, an "appalling" conclusion to the week of debates.

But what will change as a result of the new abortion legislation? That no woman takes late abortion lightly is amply borne out by the latest figures, which show that, in 1988, there were only 23 abortions between 24 and 28 weeks. Even when the bill becomes law, exceptions will be made for those women who are discovered to be carrying a handicapped child or who are themselves at risk. If MPs are serious about wanting to reduce the number of late abortions, rather than their present pastime of attempting to reduce the time-limit at every available opportunity, they could usefully make a start by declaring war on the bureaucracy and red tape that women are obliged to undergo under past and present legislation alike, known to be one of the chief reasons why late abortions are considered necessary. □

Conflict of interest case shakes NIH

- Gallo associate implicated
- Laboratory equipment link

Washington

FOR the US National Institutes of Health (NIH), which has often said that conflict of interest and scientific fraud among its scientists is not a serious problem, last week's gory exposé of a prominent AIDS researcher marked an embarrassing low point. In a three-hour hearing, congressional investigators alleged that Syed Zaki Salahuddin, a biologist in the NIH laboratory of Robert Gallo, had over six years steered nearly a million dollars of NIH business to a company of which he and his wife were secretly owners.

Investigators said that Gallo had been told four years ago about Salahuddin's possible conflict of interest, but that Salahuddin had assured Gallo that only his wife was employed by the firm and that she would leave it. Gallo apparently took Salahuddin at his word. He did not pass the information on to his superiors, according to NIH acting director William Raub.

Raub announced that NIH will now require employees reviewing contracts to sign a form stating that they have no financial interest in any contracting company. NIH is also planning to require all contractors to file a form declaring that they, too, have no conflict of interest. NIH suspended Salahuddin without pay on the morning of the hearing. The suspension will remain in effect until a separate Justice Department investigation is concluded. Representative John Dingell (Democrat, Michigan), who chairs the powerful energy and commerce oversight and investigations subcommittee, had asked the congressional General Accounting Office (GAO) to investigate the case because of concern that NIH is not doing enough to prevent conflict-of-interest abuses and is reluctant to clamp down when indications of wrongdoing arise.

According to the GAO report, Salahuddin and his wife Firoza Salahuddin co-founded Pan-Data Systems in 1984 while Salahuddin was an employee in Gallo's lab. Over the next four years, as Pan Data grew from a company worth \$921 to one with \$3 million in sales, GAO alleges that Salahuddin surreptitiously maintained his relationship with the firm. GAO says much of Pan Data's growth was due to contracts with Gallo's NIH laboratory.

In contracts with other agencies, Pan-Data benefited from Salahuddin's connection as well, says GAO. Although the company repeatedly denied to NIH that Salahuddin was connected to the

company, it told at least one other branch of the government a different story entirely. The GAO report points out that a 1985 Pan-Data contract proposal for an Army AIDS project listed Salahuddin and his wife as "corporate management personnel". In part because of Salahuddin's reputation as one of the leading retrovirologists in the field, Pan-Data won a subcontract worth \$667,514.

At the hearing, Dingell described the evidence presented by the GAO investigators as a "sorry tale of gross conflict of interest." Salahuddin, Dingell said, "created the firm, used his NIH connections to enrich the firm, did not disclose his interest as required and repeatedly lied about his role in the firm." Salahuddin, citing his Fifth Amendment rights, declined to testify. The Department of Health and Human Services Inspector General and a US grand jury are investigating the case. GAO investigators also reported that a recent NIH inventory found 184 pieces of equipment apparently missing from Gallo's laboratory.

Some of the equipment, Dingell suggested, could be found in the offices of Pan Data.

Raub admitted that, before the recent inventory, the laboratory had not been examined for five years. But it is not uncommon for NIH laboratories to share equipment, and much of the missing inventory may still be at NIH, he said.

Dingell aides say the Salahuddin case is not related to the continuing NIH investigation of Gallo's role in the discovery of the AIDS virus (see page 104). Nevertheless, the congressional scrutiny is unlikely to enhance the laboratory's reputation. Allegations were also made at the hearing that Gallo's deputy laboratory manager may have violated conflict of interest rules, by representing the Reponsif Company in a meeting with the Food and Drug Administration in 1986.

Under questioning, Raub disclosed that existing NIH reporting regulations had never exposed a case of conflict of interest.

"Either we have a situation where human beings involved in NIH contracts are not falling victim to human nature, or agency procedures for detecting such conduct are not very good", pointed out Representative Ron Wyden (Republican, Oregon). "I would like to believe the former, but the evidence seems to indicate otherwise", Raub agreed.

G. Christopher Anderson

NATURAL HISTORY MUSEUM

'Disneyland' dispute on hold for a week

London

A STRIKE over job losses at the British Natural History Museum last Friday was narrowly averted when a branch meeting of the scientists' trade union, the Institution of Professionals, Managers and Specialists (IPMS), voted to defer action pending discussions the museum's trustees.

Nevertheless, the IPMS is likely to ballot its members today (Thursday) for a mandate to take strike action in the future over job cuts proposed in the museum's corporate plan. The plan proposes a loss of 51 of its 300 science posts, but researchers have expressed concern that the museum's role as a pre-eminent taxonomic research centre would be threatened (see *Nature* 335, 4; 3 May 1990). For their part, museum director Neil Chalmers and the museum's trustees have agreed not to submit the corporate plan to the museum's parent body, the Office of Arts and Libraries, without consulting the IPMS.

Neil Chalmers was dean of science at the Open University in Milton Keynes for three years before his museum appointment.

A group of 21 senior biologists and Earth scientists at the Open University have written an open letter urging their former colleague "in the strongest possible terms" to reconsider the plan.

The plan was condemned savagely by John Evans, outgoing president of the 132-year-old Geologists' Association, addressing the association's annual general meeting last Friday. Predicting that the plan would kill off geology and palaeontology in Britain, Evans commented that the plan "has not been written in English; it has been cobbled together in American-style business jargon presumably courtesy of Disneyland where many of the museum's administration went to learn such jargon".

Henry Gee

FOSSIL REPTILE

Chipping in to keep Lizzie

London

STANLEY Wood, owner of 'Lizzie', the fossil of the earliest-known reptile which he discovered at East Kirkton in Scotland, has come down in price by £10,000, to £195,500, including tax. This will make it more affordable to the National Museums of Scotland (NMS), which must provide the money before 31 July to prevent the fossil's export to the Museum für Naturkunde in Stuttgart (see *Nature* 343, 398; 1 February 1990). Wood's gesture, which applies exclusively to NMS, matches a donation by the Geologists' Association to the NMS appeal fund. This now stands at £121,150, with £74,000 still to find — meaning that NMS must attract £902.44 per day to meet the target.

Henry Gee

Not so 'nuclear free'

Washington

UNTIL last week, the city of Oakland, California, was off limits to nuclear weapons, materials and reactors. City officials were banned from doing business with companies that participated in nuclear-weapons production, a list that included IBM, General Electric and Monsanto. Known as a nuclear-free zone, Oakland's ordinance was considered the strongest of the similar laws in 169 other US cities. But last week a California judge ruled that Oakland had gone too far. In a legal setback to the growing nuclear-free movement, the court ruled that the city's ordinance is unconstitutional.

City officials say they will appeal the decision, opening the way for a possible precedent-setting ruling by a higher court.

Federal agencies have joined the fray as well. Oakland is on a major supply route for the Lawrence Livermore National Laboratory, the main Department of Energy (DOE) nuclear weapons research facility. Several military bases located in the city also have the capability of housing nuclear weapons.

The case made headlines last year when the US Justice Department submitted an unsolicited opinion on the matter, finding that the law violated the US constitution. Local communities cannot restrict the government's ability to provide for the national defence and regulate nuclear energy, the US lawyers said.

Anti-nuclear activists charged that the administration had given in to a concerted lobbying campaign by the nation's defence contractors. In March they released leaked documents that showed that, before the justice department opinion, the Aerospace Industries Association, a Washington-based trade group, had met with the secretary of defence to urge opposition to the nuclear-free initiatives.

The association defends the meeting as its "legitimate right to meet with officials from the federal government at any time they are willing to see us". But David Birman, of the Lawyers' Committee on Nuclear Policy, feels that it is "politically very questionable" that the administration would take its first action on a nuclear-free zone (Oakland is just one of many) only after it had been lobbied by industry.

A major legal question in the case, Birman says, is whether federal law can pre-empt local law on nuclear matters. If it cannot, nuclear-free zones stand. But if federal law does take precedence, then by the same argument, international law — which bans nuclear weapons — should take even greater precedence, he says.

Next month Alameda County, which includes Lawrence Livermore Laboratory and Oakland, will vote on "Measure A", a

wide-ranging initiative that would create a government body to rid the county of all nuclear materials and research. Supporters collected 38,000 signatures last year to put the initiative on the ballot; chances are seen as good that it will pass in June. Over half of the county's residents already live in areas that voted themselves nuclear-free.

Chief target is Lawrence Livermore laboratory, which would be "converted" to purely non-nuclear work such as environmental and health research. Livermore scientists say such a move would effectively close the \$1,000 million laboratory. A third of the laboratory's current research is related to nuclear weapons, and another third has some nuclear aspect. The removal of nuclear work would be phased over a five-year period, but DOE would almost certainly sue to stop the county from tampering with the laboratory.

Defence contractors and private citizens have assembled a coalition to fight the initiative. Called the Citizens for Fiscal and Economic Responsibility, the group has already raised over a quarter of a million dollars. Last week's court decision on the Oakland ban is not expected significantly to affect the vote. But if the case goes to the Supreme Court, as some now predict it will, the Alameda initiative, as well as nuclear-free zones throughout the United States, may hang in the balance.

G. Christopher Anderson

GENOME PROJECT

Howard Hughes gets HUGO off the ground

Washington

Two years after it was founded, the Human Genome Organization (HUGO) has finally garnered its first funding of note. The Howard Hughes Medical Institute (HHMI) last week announced a \$1 million, grant, spread over four years, to support HUGO's efforts to promote and coordinate international collaboration in mapping and sequencing the human genome.

A matching grant is expected to be announced soon by Burroughs Wellcome. With over \$500,000 a year at its disposal, HUGO's first step will be to set up permanent offices (in Bethesda, Maryland, in London and in Osaka), and begin the work of helping to organize the 15-year \$2,000–\$3,000 million genome initiative.

Without major funding, HUGO has so far been more concept than reality. But genome researchers hope that new grants will finally allow HUGO to take an active role in coordinating the exchange of data, samples and technology.

G. Christopher Anderson

DFG is first to move

Frankfurt

THE West German grant agency, DFG (Deutsche Forschungsgemeinschaft) began laying out a common German research policy last week when it proposed supporting basic research in a unified Germany "from a single pot". The plan, which was unanimously approved by the DFG Senate, must still receive political approval from Bonn, East Berlin and *Länder* governments before it can take effect, probably not before 1991. DFG's constitution prevents it from providing research support for anyone outside West Germany except under special circumstances.

DFG is the first research organization in the West to announce its plans for East Germany, and if other organizations take a similar approach, the withering away of the East German Academy of Sciences is bound to follow.

DFG will stick to its principles of peer-reviewed and performance-based funding in supporting research in East Germany, according to spokeswoman Eva-Maria Streier. The consensus in the Senate, she reports, was "not to budge even half an inch" in applying the same high standards to East and West German research proposals.

Significantly, the DFG plan does not exclude applications from individual researchers in academy institutes. Although DFG generally declines grant applications from West Germans working at Max Planck institutes or GFEs (the Large Research Establishments) unless the project falls outside the normal scope of these institutes, DFG will give all applications from academy researchers a chance. But providing researchers' salaries or entire institute budgets is out of the question, she said.

The DFG plan addresses only indirectly the burning question about the fate of the academy, the largest and most important scientific institution in East Germany, whose budget of 1,000 million East German marks is due to run out at the end of this year.

Western observers estimate that between one-fourth and one-third of the academy's "research" belongs in industry or in applied research institutes similar to the West German Fraunhofer Institutes. But the fate of the other researchers — the cream of the crop in a country that has fallen far behind the West in the past ten years — is still entirely up in the air.

Dieter Simon, the chairman of the influential science advisory council Wissenschaftsrat and a permanent observer in the DFG Senate, warns that if the DFG plan is applied rigorously, "no one in East

Germany will receive anything" from DFG. University researchers, whose work has been neglected by East Germany's former Communist government, will not be able to present high-quality proposals. Without asking DFG to lower its standards, Simon calls for moderation in administering the DFG plan.

Simon urges that other organizations consider their steps carefully before acting on reunification. Recently, Research Minister Heinz Riesenhuber has come under fire from his own party for not moving faster to unify East and West German institutions. But a piecemeal approach in which the nuggets were selected by the West and the rest "dumped at the feet of the academy" would be the "worst possibility", Simon admonishes.

It would be best if the Max Planck Society and GFEs, both of which are administered by the ministry, were to wait at least until July before proceeding with reunification plans, says Simon.

Wissenschaftsrat set up a commission earlier this year to assess the potential of East German research and will consider the commission report before it makes recommendations on a new structure for West and East German science in July.

SOVIET ACADEMY

Election brings little change

Moscow

THE leadership of the Soviet Academy of Sciences will remain substantially unchanged for the next five years following the elections of 27 April, when Dr Guri Marchuk was re-elected president.

This is at odds with the frequent criticism of the academy in the Soviet press and in the scientific community, demanding major changes in the organization and management of research.

It seems now generally recognized that soviet science, including academic science, is by and large in crisis. Poor equipment, insufficient funds for fundamental research, rigid centralization of resource distribution and research planning and the effects of monopolism and totalitarianism have put Soviet science at a serious disadvantage in many areas.

Yet there have been some improvements. For example, the budget of the academy has increased 2.3 times over the past four years. But as a percentage of the national income, even this does not compare with the investment in science by leading industrial countries.

The academy has begun the switch from the financing of institutes to that of specific research programmes and projects, while the introduction of a competitive grant system is under way, but has not yet taken shape.

By world standards, the social and material status of researchers is as low as

Giving East Germany more time — possibly well beyond July, says Simon — will allow it to evaluate its own research institutions and begin to rationalize them. This would create less resentment, he says, than if "Big Brother" were to come in from the West and tell East German institutions how many people they have to let go.

Another advantage to a Wissenschaftsrat evaluation of East German science is that it gets the *Länder* involved. Until now, the *Länder*, usually major players in university policy, have been nearly silent about East German science.

Some East German Academy members are talking about breaking down the academy into a 'Leibniz Society' and a 'Helmholtz Society' for basic and applied research, respectively. But no one has emerged to take control of the academy in this critical phase. The Academy Presidium recently asked nine people if they would like to run for president of the academy. Seven declined immediately and the other two declined the next day. Three new candidates could not be found in time for the planned election in late April, so the election has had to be postponed.

Steven Dickman

ever, restricting the influx of talented young people into science. And although Soviet scientists go abroad on business far more frequently — more than twice as often as in 1985 — there is an inadequate influx of foreign scientists into Soviet institutes and laboratories, whence present anxiety about the 'brain drain'.

In organization, structure and function, the Soviet academy differs substantially from those of Western countries. It is not an assembly of elite scientists, but a whole system of institutions, laboratories, experimental production units, publishing houses and even construction facilities. Every republic, except the Russian Federation, has an academy of its own.

The USSR Academy of Sciences unites them all, coordinating research in federal programmes and areas of research. The academy's praesidium, with its staff, is in essence, a ministry of science. At present, there are about 220,000 researchers at the academy's institutes and laboratories. Yet only Academicians (they number 306) and the Corresponding Members (567) are members of the academy. Only Academicians may be elected to the leadership, and only they have the right to vote in such elections. The proposal that this right be also given to the Corresponding Members was advanced at April's general meeting, but was not even put to the vote.

The academy's scientific public is nevertheless demanding radical change.

A Scientists' Union of the USSR has been formed, together with republic and regional unions, but they are still taking shape and their influence is as yet limited. Some hopes for radical change were linked with the academy elections last month and they, as required by the amended statutes of the academy, were indeed more democratic than previously.

For the first time, there were no instructions of which candidates were approved or 'recommended' by higher-ups. There were six nominees for the post of president — two scientist members of the presidential council, Stanislav Shatalin and Yuri Osipyan, together with Academicians Guri Marchuk, Nikolai Basov, Zhores Alferov and Andrei Gaponov-Grekhov. But all of them except Marchuk, who has held the post since 1985, withdrew. Of the 247 Academicians who were present at the meeting, 195 voted for Marchuk and 43 against.

By contrast, there was no choice in the elections of the vice-presidents and of the chief scientific secretary. (On the proposal of the president, Academician Igor Makarov holds on to that post.) The election of academicians as secretaries of the academy's departments, as well as of other members of the praesidium, evoked little debate.

All the vice-presidents in charge of particular fields of research have retained their posts. They are Academicians Vladimir Kudryavtsov (social sciences), Yuri Osipyan (physics-mathematical sciences), Yevgeny Velikhov (information science and energy), Konstantin Frolov (machine-building, mechanics and control processes), Oleg Nefedov (chemical sciences), Rem Petrov (biological sciences), Nikolai Laverov (Earth sciences, also chairman of the USSR State Committee for Science and Technology, and, in this capacity, vice-chairman of the USSR Council of Ministers) and rector of Moscow State University Anatoly Logunov (organization of research-training centres and publishing).

Three other vice-presidents represent the major regional academic organizations — chairman of the Siberian department Valentin Koptug; the Ural department Gennady Mesyatz; and the Leningrad Research Centre, Zhores Alferov. (The Leningrad Centre, which comprises over 30 research institutions, has been given this status for the first time.)

The post of vice-president for the Far Eastern department of the USSR Academy of Sciences, where neither Academician Viktor Ilyichev (the former head of the department) nor the other two contenders won a majority vote, remains vacant. This question was not considered at the April meeting for lack of recommended candidates, but the situation there is considered bad.

Yuri Kanin/Novosti

Allegation of insider dealing

Washington

GENENTECH, the leading US biotechnology company, disclosed last week that the Securities and Exchange Commission (SEC) was investigating a possible case of insider trading that took place before the announcement, on 2 February, of the company's proposed \$2,100 million merger with healthcare conglomerate, Roche Holdings (see *Nature* 343, 495; 1990).

The SEC inquiry, sparked off by an unusually high volume of trading in Genentech securities on the Pacific Stock Exchange just before details of the merger were made public, focuses on the wife of G. Kirk Raab, the company's president and chief executive officer. In particular, it concerns certain 'communications' between Molly Raab and a member of her family, and on subsequent trading in Genentech securities by persons other than herself.

In a short statement issued by the company, Genentech makes it clear that it believes that neither it nor Mr Raab is the subject of any investigation and that Mr Raab exercised proper care in maintaining the confidentiality of the merger information. Mr and Mrs Raab, who are said to be cooperating fully with the investigation, would not comment while the inquiry is in process.

Brief mention of the inquiry was made in the detailed proxy statement outlining the merger agreement, which was sent to shareholders last week. Shareholders will be asked to vote on the proposed merger with Roche at an annual meeting of stockholders, postponed since April, and now due to be held on 8 June. Stock analyst Stuart Weisbrod believes this inquiry "is not going to affect the deal [merger] at all".

Interestingly, the proxy statement reveals that although Roche will have only two members on a 13-member board of directors, it will wield somewhat more influence over Genentech's business transactions than was originally anticipated. Roche is to have the power of veto over acquisitions or the sale of assets and over the issuing or repurchase of the company's securities. In addition, Genentech cannot enter into licensing agreements with respect to its products or technologies without offering Roche first refusal.

Weisbrod notes that the "unusually high" severance awards — so called 'golden parachute awards' — payable to the top six Genentech officials should they lose their jobs as a result of the merger, would be "the only thing that shareholders at the meeting can question". Under these agreements, chairman Robert A. Swanson and Raab would receive payments of \$6.3 million and \$6.6 million respectively.

When asked whether such lump sum severance awards equal to five times the employee's salary and bonus were unusually high, Genentech spokesperson Susan Rogers said that these people "have put their positions on the line" and that these separate agreements are intended

BIOTECHNOLOGY

Little apparent interest in Celltech sell-off

London

A BUY-OUT of Celltech, Britain's largest biotechnology company, by a large pharmaceuticals company now seems unlikely. The possibility emerged last autumn, when British and Commonwealth Holdings (B&C), decided to sell its majority 36.4 per cent stake, providing a golden opportunity for a takeover attempt (see *Nature* 342, 334, 23 November 1989). But John Jackson, Celltech's chairman, last week confirmed that an announcement that the company is looking for a new chief executive means that no single buyer has emerged and the B&C shares are likely to go to smaller investors.

A German or Japanese buy-out of Celltech was expected by some analysts, a possibility that provoked alarm in the United Kingdom. Labour opposition leader Neil Kinnock, speaking to the Parliamentary and Scientific Committee in February, criticized the proposed sale, because of Celltech's involvement with the government-funded UK Medical Research Council. The company has a 'memorandum of understanding' with the council to exchange research information.

Other analysts are not surprised by the turn of events. Although Celltech's expertise in monoclonal antibody technology might be of interest, Celltech has not yet matured financially into a ripe takeover target, they say. The company was formed in 1980, and recorded its first profitable year in 1987.

Mark Dodgson, from the University of Sussex, who has spent a year studying Celltech, describes the company's predicament as "a tragedy". He says that Celltech's expensive research and development programme relies on long-term investment. B&C's withdrawal means that Celltech has to look to a venture capital market for finance which tends to view investments on their short-term merit.

B&C's Celltech shares may even end up being disposed of by an official receiver, because liquidation of B&C is now a possibility. The company is reeling following the collapse of Atlantic Computers, B&C's computer leasing subsidiary.

Peter Aldhous

"to protect them personally in the event of any adverse consequences".

Even if shareholders approve the merger in June, the effective date of merger could be delayed if at that time clearance by the Federal Trade Commission has not been given. Genentech and Roche have still to satisfy the Hart-Scott-Rodino Antitrust Improvements Act.

Diane Gershon

ARCTIC RESEARCH

Arctic cooperation

Boston

A DRAMATIC increase in the number of cooperative international agreements governing Arctic research and affairs is documented in a report released by a presidential advisory group the US Arctic Research Commission. The report cites some 450 active governmental and scientific agreements worldwide that pertain to the region, more than half of which have been reached within the past decade, suggesting that Arctic research is beginning to garner the kind of politically active global scientific constituency that Antarctic research has enjoyed for some time.

The surge in international arrangements is partly due to increasing interest in research on global environmental change; the environmentally sensitive Arctic region may provide important early indications of global climate change, atmospheric pollution and greenhouse warming. The role of the Arctic Ocean in global change processes is coming under particular scrutiny — it is known to influence the deep water of the North Atlantic, one of the dominant water masses in the earth's oceans. The Arctic Ocean is thought also to play a disproportionately significant role in the removal of biogenic and human-made materials from the atmosphere and is an important "heat sink" for the global atmospheric system. But, Arctic researchers say, we know less about the Arctic Ocean than any other ocean in the world.

The report also highlights an increase in the number of cooperations with the Soviet Union. Soviet willingness to participate in international efforts, especially those concerning its vast territorial holdings in the East Arctic, has helped to account for what the report terms a "geometric" increase in international cooperative agreements in the region. Soviet interest has not been limited to research on global change: recent bilateral and multilateral agreements govern everything from the protection of polar bears to contingency plans for oil spills or radioactive accidents in the region.

Seth Shulman

"Preliminary International Agreements for Research, Logistics and Access Concerning the Arctic", is published by the U.S. Arctic Research Commission, Washington, DC, April 1990.

HIGH-TEMPERATURE SUPERCONDUCTIVITY

US exposes same old faults

Washington

As visions of levitated trains and cheap electricity from superconducting generators have faded, research into high-temperature superconductivity, now four years old, has settled in for the long haul. But the initial ballyhoo caught the imagination of politicians as well as the public in the United States, and coming along with fears of Japan's rising technical prowess, it propelled the notion that high-temperature superconductivity could be the vehicle for new policies that might help turn basic research into marketable products. Now, says a report from the congressional Office of Technology Assessment (OTA), the research is going well but the policy derailed some time ago.

Total spending on high-temperature superconductivity research in the United States and Japan is about the same. In 1989, the US government spent \$130 million, and the Japanese about \$70 million, but for spending by industry the figures are almost reversed. The quality of the research seems to be on a par: the OTA report describes US research as "second to none", while in the March 1990 issue of *Science Watch* two of three 'hottest' (most cited) papers in physics concern high-temperature superconductivity and both from Japan. But although equal now, differences are set to emerge.

Almost half the high-temperature superconductivity money from the US government has gone to the Department of Defense, while Japan's spending is all aimed at commercial, not military application. In addition, the remainder of the US

federal research support goes predominantly to the national laboratories, which have a poor track record in technology transfer to industry.

In Japan, a big success by MITI (Ministry of International Trade and Industry) was the establishment in 1988 of ISTEC (the International Superconductivity Technology Center), a consortium of industrial contributors (including a handful of US and European companies) who each pay about \$100,000 annually to support high-temperature superconductivity research. Although ISTEC is the kind of government-led effort that generally inspires fear and envy in the United States, the OTA report plays down its significance, arguing that it is occupied entirely with basic research of the kind that is adequately supported, but in different ways, by the US government.

The bigger threat to US supremacy, according to OTA, is the much greater level of support by Japanese industry, and the more patient thinking that lies behind it. In a survey of US and Japanese industries doing high-temperature superconductivity research, US respondents said, on average, that they expected to see the first commercial products on the market in 1992, whereas the Japanese companies saw the likely date as 2000. Given the fairly equal base of research in the two countries, the difference is presumed to reflect more favourably on Japanese persistence than US optimism.

David Lindley

High-Temperature Superconductivity in Perspective: OTA-E-440, April 1990.

JAPANESE TECHNOLOGY

Fuzzy computers go home

Tokyo

JAPANESE housewives are about to have their first encounter with fuzzy computing. Matsushita Electric Industrial Co. has announced the launch of two new products intended to ease household cleaning chores, the fuzzy vacuum cleaner and the fuzzy washing machine. These are the first consumer products to emerge from a huge effort by Japanese industry to develop fuzzy computing technology.

Fuzzy computing is based on fuzzy set theory, developed by L. A. Zadeh of the University of California in the 1960s. Whereas conventional computers operate on Yes/No logic, fuzzy computers form conclusions from imprecise data (such as 'faster' or 'stronger') by giving such input a value somewhere in the continuum between zero and one.

Matsushita's fuzzy washing machine, affectionately called 'Aisaigo (beloved wife) Day Fuzzy', comes equipped with

two 'eyes' (optical sensors) which can detect the quantity of clothes and the quality and quantity of their dirt. A fuzzy microcomputer then selects the most suitable of 600 possible cycles to wash the clothes. The fuzzy vacuum cleaner assesses the amount of dust and the nature of the floor and adjusts the suction power of the cleaner accordingly. Both machines are intended to allow housewives "to enjoy easier, more comfortable lives", says a company press release.

Matsushita is just one of many Japanese companies involved in developing fuzzy computing. Last year, MITI set up the Laboratory for International Fuzzy Engineering Research (LIFE) with the backing of 42 companies, including steel, automobile, electronics, transportation, electric utility and security companies. And the Tokyo stock market is a future target for fuzzy control through fuzzy programme trading.

David Swinbanks

ENVIRONMENTAL ACTION

Banking on greener legislation

Washington

MORE than 200 legislators from 42 countries outlined a broad legislative strategy to combat global environmental problems at a three-day meeting in Washington last week. Hosted by the US Congress, the Interparliamentary Conference on the Global Environment was intended to co-ordinate environmental legislation around the world. The lawmakers called for a "global Marshall plan" to help developing countries deal with their environmental problems without undermining their economic growth.

They also proposed a "green common market" through which industrialized nations could share technology and agricultural techniques that can reduce greenhouse gas emissions. A "bank for sustainable development" could lend money to developing countries for projects that would not harm the environment. The legislators also called for a 50 per cent reduction in greenhouse gases by the year 2010. Although the conference delegates do not make policy for their governments, several said they would soon introduce legislation in line with the conference resolutions.

G. Christopher Anderson

GLOBAL WARMING

Majority verdict on temperature rise

Washington

MOST climate change scientists believe that a global temperature rise of 2 degrees Celsius or more over the next hundred years is a better than even chance, according to an international survey released last week by the Global Environmental Change Report, an Arlington, Massachusetts-based newsletter. But a majority of the scientists who replied to the survey admitted that scientific evidence currently available cannot yet prove the case.

The newsletter sent out 1,500 questionnaires to a random sample of scientists studying climate change. Of the 330 that returned the survey, 65 per cent believe that the two-or-more degree temperature rise is 50-100 per cent likely; 31 per cent think it has less than a 50 per cent chance.

Over 93 per cent of the scientists questioned believe that some significant warming will occur over the next century. Although 76 per cent think that human-induced greenhouse warming is already occurring, only 41 per cent believe that science can substantiate that opinion. However, nearly 90 per cent think that countries should take immediate action to reduce CO₂ emissions, despite the scientific uncertainties that remain.

G. Christopher Anderson

Time not right for Gallo

Washington & Langen, West Germany

A PRESS conference at which Robert Gallo of the US National Cancer Institute was set to give his side of the dispute over who was first to discover the AIDS virus was abruptly cancelled last week after Gallo decided that "a story about this now would not help science".

The conference was to have been held at the newly opened Paul Ehrlich Institute in Langen where Gallo was attending a scientific meeting. Gallo said he wanted to clear his name in West Germany where the press had repeated statements from an article in the French newspaper *Le Monde* which quoted Luc Montagnier of the Pasteur Institute as appealing to Gallo to "at last accept the evidence" and admit the Pasteur Institute as the true source of the AIDS virus.

In addition to enraging Gallo, Montagnier's statements may breach a 1987 treaty between France and the United States (see *Nature* 344, 481; 5 April 1990) which named Gallo and Montagnier as "co-discoverers" of the AIDS virus and forbade both Gallo and Montagnier from contradicting the "scientific history" laid out in the treaty.

Gallo also said that he decided not to hold the press conference because he did not want to prejudice the inquiry being conducted by the US National Institutes of Health (NIH). Gallo said that he would be more than willing to talk "in a few weeks." First, he said, "I must go through this process to its completion. Then I will show you my material." The NIH inquiry (which Gallo insists is "interviews" rather than "investigation") was stimulated by a 16-page article by John Crewdson in the *Chicago Tribune* newspaper which repeated allegations that the AIDS virus isolated by Gallo may have come from samples provided by Montagnier. According to a letter from NIH to the US Congressional Subcommittee on Oversight and Investigations, NIH is "assembling and analyzing information" relevant to issues raised in Crewdson's article.

Underpinning these issues is the key question of whether the virus isolated in Gallo's laboratory at the end of 1983, then known as HTLV-3, is the same as that isolated from a French AIDS patient by Montagnier earlier that year. Montagnier sent a sample of his virus, designated LAV, to Gallo in September 1983 and, in a patent dispute in 1985, claimed that Gallo had used LAV grown from that sample to develop an antibody test.

Against the now known background of genetic variability of the virus, the published genetic sequences of HTLV-3 and LAV are remarkably similar, with no two isolates having exactly the same genetic make-up. The virus also mutates rapidly

in infected patients, so viral isolates taken from one patient at different times may differ markedly. This fuelled suspicion about the source of HTLV-3.

The sequence similarity led Crewdson to conclude that Gallo obtained HTLV-3 from cultures of LAV, either deliberately or in error. According to Crewdson, Gallo has always maintained that LAV was not grown successfully in his laboratory. But from his analysis of laboratory notebooks and memos sent to Gallo from his chief virologist, Crewdson traces a route — through many gaps in the record — by which LAV could have been grown for long periods of time in the laboratory, eventually re-emerging as HTLV-3.

Crewdson alleges that Gallo knew about LAV's relationship to AIDS before he published his own discovery of HTLV-3 in 1984. This, and questions of exactly when Gallo's researchers first embarked on isolating HTLV-3, are the focus of the NIH inquiry:

■ According to Crewdson, Gallo, in a sworn statement to the patent committee in 1985, said that before the patent for his antibody test was granted in May 1985, he had seen no evidence linking LAV to AIDS. But Crewdson contends that by April 1984, Gallo had been told by the Pasteur group that its LAV-based antibody test worked as well in trials conducted by the US Centres for Disease Control as his own test developed from HTLV-3.

EPIDEMIOLOGY

New data to reveal leukaemia links?

London

THE factors underlying the occurrence of localized clusters of leukemia cases will come in for further scrutiny following the announcement of the results of a survey that shows such clusters are uncommon in England and Wales.

The survey, published yesterday, is the work of Ray Cartwright and colleagues at the Leukaemia Research Fund (LRF)'s clinical epidemiology centre in Leeds. It catalogues the distribution of leukaemia and lymphoma cases from 1984 to 1988 among a population of 15 million people.

The authors do not attempt to explain the occurrence of individual clusters, such as the tenfold excess of cases near the Sellafield nuclear plant. In the absence of a general pattern of clustering, epidemiologists may be justified in looking in detail for causes of particular clusters. Competing theories include the Gardner hypothesis that high paternal radiation doses may cause leukaemia in children (see *Nature* 343, 679; 22 February 1990) and variants of the theory that viruses are to blame.

The new survey is the most comprehensive to date of leukaemia incidence in the

■ Crewdson claims that, in 1983, Gallo's researchers themselves discovered that HTLV-3 and LAV are immunologically similar, but did not publish the results. Despite Gallo's denial to Crewdson that detailed studies of LAV were carried out in his lab, Gallo published a letter in *Science* showing electron micrographs of LAV taken at that time, one of which had been inadvertently labelled as HTLV-3 in an earlier *Science* paper.

■ In a letter to *Nature* in 1986, Gallo claimed that he obtained the first isolates of HTLV-3 well before September 1983, when the shipment of LAV arrived in his laboratory. Crewdson notes inconsistencies between data in that letter and in an earlier paper by Gallo.

One issue that NIH has specifically said it will investigate is a deletion in a letter obtained by James Swire, a lawyer representing the Pasteur Institute. The letter had been written by an electron microscopist who had been studying cells containing viruses from Gallo's laboratory. According to Crewdson, the original version of the letter said that the French virus had been successfully photographed growing in a type of T cell that Gallo claimed had never been used for culturing LAV in his laboratory. But when, in response to a Freedom of Information Act request in December 1985, a copy of the letter was sent to Swire, Crewdson claims that all mention of LAV had been deleted.

The NIH committee of inquiry is expected to conclude by mid-summer.

David Concar & Steven Dickman

United Kingdom, and gives cancer epidemiologists a sound database to link leukaemia incidence with putative causal factors. The LRF team are now looking at a number of possibilities, including the correlation of indoor radon exposure with the distribution of the diseases. In the survey, they note that two forms of leukaemia "display a tendency towards high rates in the extreme south-west", where indoor radon levels are the highest in the United Kingdom.

Their data should shed light on the controversial link between exposure to radioactive radon gas and leukaemia, suggested by a team of physicists from Bristol University (see *Nature* 345, 4; 3 May 1990). The Bristol study correlated average indoor radon concentrations with cancer records across 14 countries. But in some cases, the geographical areas covered by the cancer records and radon data did not overlap completely. The LRF data will allow a finer-grained correlation for better defined geographical areas.

Peter Aldhous

"Leukaemia and Lymphoma: an atlas of distribution within areas of England and Wales 1984-1988" is published by the Leukaemia Research Fund.

NUCLEAR RESEARCH

Waste issue threatens reactor

Munich

EVEN a small nuclear reactor can stir up a large controversy in West Germany. This truism, which became even truer after Chernobyl, is being demonstrated once again in West Berlin, as a political dispute over waste disposal threatens licensing of the research reactor at the Hahn–Meitner Institute (HMI), the city's only Large Research Establishment.

An article in the magazine *Spiegel* on 23 April declared that spent fuel rods from six West German research reactors, including the one at HMI, consist of 93 per cent uranium-235 — “pure bomb material”. *Spiegel* says that uranium from the fuel rods, which have in the past been returned to the United States after use, may wind up in US nuclear bombs.

HMI spokesman Thomas Robertson rejects SPD's arguments as “nonsense” for two reasons. He says that buying US fuel rods for the reactor and returning them when they are spent removes uranium-235 from the nuclear fuel cycle, rather than adding it, and that plutonium in the rods would be too strongly contaminated to serve as raw material for a bomb. Furthermore, he adds, the United States already has so much weapons material that it would be irrelevant whether these few fuel rods added to it.

Nevertheless, West Berlin Senator Barbara Riedmüller-Seel (Social Democrat, SPD) sent a letter to West German Research Minister Heinz Riesenhuber (Christian Democrat) asking the Research Ministry, as 90 per cent shareholder in HMI, to promise that the spent fuel rods were not being recycled into bombs.

Robertson doubts that Riesenhuber could give the assurance that SPD has asked for.

The letter from Riedmüller-Seel seems to represent a turnabout in the West Berlin SPD's grudging acceptance of the necessity of licensing the reactor. The HMI reactor was shut down three years ago for upgrading to a more powerful neutron source, but in the meantime there have been such grave problems with waste transport and disposal in the United States that HMI signed a new contract for waste disposal with the Dounreay facility in Britain.

Robertson admits that fuel from research reactors is not kept separate from bomb material at Dounreay, but he asserts that the Environment Ministry in Bonn, which is also responsible for reactor safety, has approved of the Dounreay waste disposal plan.

Squabbles over the licensing procedure have several times brought the fragile coalition between the SPD and the anti-nuclear Alternative List, the local branch

of the Green Party, to the brink of collapse (see *Nature* **341**, 479; 1989). But the SPD may be trying to have its cake and eat it too — trying to please its anti-nuclear supporters while at the same time declaring its support for the reactor. A spokeswoman for Riedmüller-Seel, Sigrid Kneist, says that the letter to Riesenhuber was not an attempt to delay the licensing, which SPD has promised by the end of May.

Kneist says that the West Berlin government realizes how high the stakes are: Siegfried Grossmann, the nominee for the vacant directorship of HMI, has

SOVIET SCIENCE

US Academy describes anti-semitism

Washington

AMID reports of rising anti-semitism in the Soviet Union, the US National Academy of Sciences recently urged the Soviet authorities to condemn anti-semitic attacks and persecution. The two-paragraph resolution, adopted at the 127th annual meeting of the body, notes cases of “harassment or worse”, and asks responsible authorities to “use all available means to prevent their further occurrence”. Many Jewish scientists in the Soviet Union are concerned about the threat of persecution and even pogroms, according to former Soviet scientist Valery Soyfer, a molecular biologist at Ohio State University who has been monitoring reports of anti-semitism in the Soviet Union. He says one recent lecturer at a Soviet science centre, discussing the Jewish influence in the country, told his audience that machine nuts in the Soviet Union have six sides, rather than four, because of “Zionist propaganda”. “Even inside academic institutions there are such battles”, Soyfer says.

G. Christopher Anderson

NUCLEAR INDUSTRY

New order for BNFL

London

BRITISH Nuclear Fuels plc (BNFL), shaken recently by the Gardner report's association between childhood leukaemia and paternal radiation exposure at the Sellafield nuclear reprocessing plant, has welcomed a £225 million contract to reprocess 450 tonnes of reactor fuel from Lingen, West Germany.

The contract is the first to be signed for reprocessing in the twenty-first century at BNFL's Thermal Oxide Reprocessing Plant (due to open at Sellafield some time in 1992).

With the future development of Britain's nuclear programme in abeyance until 1994, overseas contracts are set to become

said that he will take the job only if the licensing proceeds on schedule. The West Berlin Senate was planning to discuss the issue on 8 May.

Depending on Riesenhuber's response, the row may have dramatic consequences for other research reactors in West Germany at Jülich, Garching and elsewhere. The problem may resurface in an especially acute way in Garching, near Munich, where a project to build a new research reactor on the Technical University campus has been stalled for several years, in part because the Bavarian government feared a local backlash from opponents of nuclear energy.

Steven Dickman

UK SPACE SCIENCE

Optimism, but little money

London

ANNOUNCING approval from the Science and Engineering Research Council for a £17 million British involvement in two space projects, Professor Len Culhane from the Mullard Space Science Laboratory added that the climate for British space science was “substantially improved” after financial cuts in the 1980s. But this optimism, it seems, reflects a better state of mind, not increased funding. As Professor John Harries, head of space science at the Rutherford Appleton Laboratory, commented: “It's nice when someone stops hitting you”.

The new money is for British teams to develop equipment for X-ray and ultraviolet astronomy missions. The X-ray multi-mirror mission, a ‘cornerstone’ of the European Space Agency's Horizon 2000 space programme, is due for launch in 1998.

Lyman-FUSE, a joint project with the United States and Canada to look at signals in the far and extreme ultraviolet, will fly in 1997.

Peter Aldhous

a more important part of BNFL's business.

Also at the end of last month, the Health and Safety Executive's Nuclear Installations Inspectorate approved plans for BNFL to operate its nuclear reactors at Chapelcross in Scotland and Calder Hall in Cumbria until 40 years old, subject to an estimated £20 million worth of safety improvements.

The reactors were designed with a 30 year lifespan, which they have now reached. Although reactors at both sites supply plutonium for Britain's nuclear weapons programme, BNFL say that the decision to continue operation is due to the reactors' commercial value as electricity generators.

Peter Aldhous

HUGO physical mapping

SIR—HUGO, the international Human Genome organization, has adopted as one of its main objectives the coordination of physical mapping efforts on individual chromosomes. It is our desire to sponsor, on a chromosome-by-chromosome basis, committees, workshops and scientific meetings aimed at facilitating exchanges of data, samples and materials and optimizing the integration of individual laboratory efforts. HUGO will also help to coordinate these evolving physical mapping efforts with the existing chromosome committees of the Human Gene Mapping Workshops. HUGO staff will be able to provide guidelines for committees, workshops and meetings on the basis of past experience. It is ready to aid in the planning and organization of these meetings, including assistance in the preparation and submission of applications for funds to support them. In addition, HUGO has some funds of its own which may be available to supplement support received from other sources.

Individuals currently involved, or interested in participating in organizing efforts on particular chromosomes should contact any one of the three regional HUGO offices listed below*. It is expected that these three offices will share the overall administrative burden for physical mapping, with particular offices playing a leading role for individual chromosomes as appropriate.

Several guiding principles will determine HUGO's role in selecting particular chromosome activities for support and encouragement. HUGO is committed to the principle of free exchange of materials and information. Only one organized group for each chromosome can receive official HUGO sponsorship. However, this group need not have a single lead organization or individual, provided that there is a suitable plan for collective management representing the bulk of the researchers interested in that particular chromosome. HUGO will maintain a summary of existing coordination activities on each chromosome.

HUGO will act as an information exchange centre for communication among the individual chromosome efforts. It will provide working examples of effectively organized data sharing, sample sharing, workshops and committee structure as these emerge. At present (during the current period of rapid changes in technologies and strategies), there is no consensus on how physical mapping activity should be organized or implemented. Coordination of these efforts, through HUGO, after an initial period of experimentation should enable a better definition of global strategies and procedures to be

developed over the next few years.

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Radiation doses

SIR—There are some serious faults in the paper by Gardner *et al.*¹ claiming that four of the cases of child leukaemia out of 46 cases studied were due to radiation doses of 100 mSv or more received over 6–10 years by their fathers before the child was conceived, producing a heritable tendency to leukaemia.

No excess of leukaemias or of any other heritable diseases has been observed among 7,400 children of the irradiated survivors of Hiroshima and Nagasaki (5 leukaemias observed, 5.2 expected for an unirradiated but otherwise similar group). The natural background dose rates in Kerala in India and in parts of Brazil are close to the dose rate received at Sellafield by the fathers concerned, and no extra leukaemias have been reported among the offspring of male visitors to these areas.

Contrary to popular belief, hardly any of the recorded clusters of child leukaemias in Britain have any connection at all with ionizing radiation. The 165 child leukaemia deaths on Tyneside² for example can be explained only as the effect of an infective agent, probably a virus; and at the other end of the scale the same applies to the three leukaemias that occurred in the United States in a single house between 1958 and 1965³.

The strongest reason for rejecting the Gardner hypothesis is that a far simpler explanation of child leukaemia clusters has been suggested by Kinlen⁴. He suggested that the frequent association of leukaemia with nuclear and other large establishments arose when a large influx of population from elsewhere, some of them carrying a virus to which they themselves were immune, was added to a small isolated community in which the children were not immune. Strong support was gained for his theory when he found that the small, excess-radiation-free and relatively isolated Scottish town of Glenrothes had had a rapid and large increase of population from elsewhere — and had then experienced a leukaemia epidemic among the children of the earlier inhabi-

tants. This theory was widely accepted as an adequate reason for the Seascale leukaemias without any contribution from ionizing radiation.

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2. *The Listener* 26 November 1987.
3. Garfinkel, L. *CA-A Cancer J. for Clinicians* **37**, No. 1, 20 (1987).
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SIR—In the wake of the Gardner report (see *Nature* **343**, 679; 1990), the British nuclear industry might usefully adopt the guidance given in the Control of Substances Hazardous to Health (COSHH, 1988) Regulations, which came into force on 1 October 1989.

Section 7 (1) of the COSHH Regulations states that “every employer shall ensure that the exposure of his employees to substances hazardous to health is either prevented or, where this is not reasonably practicable, adequately controlled”. This should apply to both toxic and radioactively hazardous materials.

Furthermore, Section 12 (1) stipulates that “an employer who undertakes work which may expose his employees to substances hazardous to health should provide that employee with such information, instruction and training as is suitable and sufficient for him to know: (a) the risks to health created by such exposure; and (b) the precautions which should be taken”.

Based on interviews with Sellafield workers broadcast and reported in the wake of the Gardner report, I judge that British Nuclear Fuels plc's health physics expertise has not on all occasions been shared fully with their workforce.

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Eminent engineers

SIR—John Maddox, in ‘Engineer in the White House’ (*Nature* **344**, 103; 1990), says that “Mr John Sununu, Chief of Staff at President George Bush's White House, is probably the first person with a technical background in such an influential position since Benjamin Franklin officiated at the court of George Washington”.

It is questionable whether Franklin ‘officiated at the court of George Washington’, but it is a fact that Presidents Herbert Hoover and Jimmy Carter were both engineers.

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Another view of Vavilov

SIR—There has been a tendency of late to lay the blame for Lysenko's ascent at N. I. Vavilov's door. Valery Soyfer is definitely of this opinion. I should like to draw attention to just a few of the discrepancies in his article "New light on the Lysenko era" (*Nature* 339, 415–420; 1989) which make it difficult to accept his view.

Let me begin with the All-Union Congress of Genetics, Plant and Animal Breeding held in Leningrad on 10–16 January 1929, where Lysenko first appeared before a representative forum of Soviet and foreign scientists. Soyfer makes much of the fact that Lysenko received an invitation to the congress and regards it as evidence of Vavilov's patronage (Vavilov was president of the congress). Yet in the congress proceedings (Vol. 1, pp 16–78) the names, addresses, place of work and occupation of all 1,500 or so of the participants are given. The Gandzha Plant Breeding Station, where Lysenko worked as senior specialist, was represented by a delegation of 21 people, from laboratory assistant to director; all of them, presumably, received the "prestigious invitation".

In his closing speech, Vavilov stressed that the congress was, indeed, an All-Union one, in which every republic and all organizations doing research in genetics, plant breeding and animal breeding were represented. He made special mention of the fact that young researchers had taken an active part in the organization and in the work of the congress (*Proceedings* Vol. 1, pp 125–128).

Taking for granted that Vavilov played the chief role in Lysenko's advancement, Soyfer puts forward the following explanation for his motives. Vavilov, as director of the Institute of Applied Botany and New Cultures, had gathered a collection of seeds of wild and cultivated plants. He intended to use the collection to introduce new varieties of cultivated plants by transferring valuable genes to strains extant in the Soviet Union. But he did not know how to go about it (a strange supposition given that he had such outstanding scientists as plant physiologist N. A. Maximov and plant geneticist G. D. Karpechenko working in his institute). Soyfer writes: "Not himself an expert on plant breeding and without much experience of fieldwork, Vavilov could only hope the plan was feasible." The story goes that Vavilov and his colleagues encountered problems that could not be easily solved because of the differences in physiological requirements between foreign and local plants, when suddenly there came a man, Lysenko, who could solve a more complex problem — the conversion of winter crops into spring crops by cold treatment! Hence the "prestigious invitation" (to-

gether with 20 other specialists of the Ganzha station). For over 60 years the world scientific community has been labouring under the delusion that Vavilov was one of the great scientists of the twentieth century, and an expert in plant breeding and fieldwork. Quite wrongly, Soyfer portrays Vavilov as a sort of babe in the wood, mesmerized by Lysenko and his work.

To "confirm this interpretation", Soyfer refers to an interview given by Vavilov to the Leningrad newspaper *Smena*, published on 11 January, in which he supposedly spoke of the idea of 'transformation' (whatever they may mean). But the word 'transformation' was not even used. *Smena* featured several brief interviews with Vavilov and with a number of the congress participants, including the foreign guests. But there was no allusion to Lysenko or his work whatever.

Soyfer then points to an article in the newspaper *Sotsialisticheskoye zemledelie* (*Socialist Agriculture*) as though it were written by Vavilov himself — he gives the reference as "Vavilov, N. I. *Sotsial. zemledelie*, No 54 (616), p. 1 (24 February 1931)". But that was not an article by Vavilov. It was an account of Lysenko's report about his work in 1930, which he made at a meeting of the presidium of the All-Union Academy of Agricultural Sciences, and the decision made by the presidium.

As further 'evidence' Soyfer selectively quotes a passage from *Socialist Agriculture* of 13 September 1931, an account of a meeting of the State Commissariat for Agriculture at which Vavilov spoke. But he cites only the part where Lysenko's work is favourably mentioned. This is the quotation:

Of special interest are the works of Lysenko who concretely approached [the problem] of changing late-ripening varieties into early-ripening ones, of transforming winter varieties into spring varieties. The facts he established are indisputable and of considerable interest. *But it must be definitely said that tremendous collective work is required in order to elaborate concrete effective measures by means of which the vegetative period could be changed for practical purposes.*

The sentence in italics was omitted.

According to Soyfer, "Only in 1936–37 did Vavilov realize what sort of person his protégé was". To support this contention, Soyfer had to find some favourable remarks about Lysenko's works that Vavilov had made at an earlier date. The reader is referred to what Vavilov said, supposedly in 1934, at a conference for planning genetics and breeding research. The reference given is: "Vavilov, N. I. Theoretical principles of the selection of plants. Moscow-Leningrad, Vol. 1,

p. 865 (1935)". But the conference was held on 25–29 July 1932, and the proceedings were published in 1933. Moreover, the work cited is a collection of articles in three volumes edited by Vavilov. Volume 1 features four long articles by Vavilov himself, but in none of them is Lysenko mentioned. The quotation used by Soyfer in fact comes from an article "Problems of the vegetative period" by L. P. Basova *et al.*, who refer to Vavilov's report at the conference. This is a curious way of citing an author — not only out of context, but out of somebody else's paper. So much for the "new light on the Lysenko era" as far as Vavilov is concerned.

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Ivory poaching

SIR—I have recently returned from a hydrogeological mission to the arid and semiarid provinces of Kenya and have become better acquainted with the problem of the extermination of the elephants by the ivory poachers.

There are many measures to be taken and the Kenyan government is doing its best. After touring the areas through which the poachers come and smuggle the ivory, I can understand the tremendous difficulties of solving the problem. These areas are very scarcely populated by nomadic people and people coming from over the borders exploit this fact. They are also very well equipped to take their booty and disappear.

I would like to suggest some additional measures in order to save the elephants.

(1) Immediate measures: A special UN force should be mobilized in order to help the government to cope with the problem of poaching. I am sure that many volunteers from all over the world would be happy to join this force.

(2) Intermediate measures: Transfer elephants, in their thousands, to inland regions where they can be better protected from the poachers. This has to be carried out as an international effort, something like that mounted when the Aswan dam in Egypt was built and the archeological monuments were transferred. Such areas of refuge exist in the central Rift Valley provinces. Some of these areas are scarcely inhabited today.

(3) Long-term measures: Start an international programme of water development in the arid and semiarid regions bordering the savanas to give the people a safer and better source of income, thus eliminating the main cause for their need to take the risk of poaching.

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Reflections of science as a product

Judah L. Rosner

A new form of title for scientific reports, one which confidently asserts a conclusion rather than implying it, is becoming more prevalent. That's a bad thing.

THE success of molecular biology has led to the expectation that it will continue to deliver answers to basic questions and to provide medical and commercial products. The status and rewards that can nowadays be won by molecular biologists also mean they have to convince journals and funding agencies that their particular line of work is important, even definitive. Have the stakes involved influenced scientists to compromise on their traditional values?

One indication that they have is to be found in the trend of using a particular type of title in scientific reports. The new form is the assertive sentence title (AST). ASTs differ from traditional titles in that they are not clauses but complete sentences that assert a conclusion. "DNA is the Genetic Material" is an AST; "DNA as the Genetic Material" and "DNA, the Genetic Material" are not, nor are questions. The AST has the form of an eternal truth, whereas the traditional choice of words does not state (although it may imply) a conclusion.

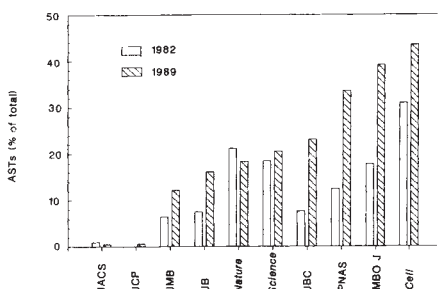
Out of a total of 160 titles reprinted in several 'classic papers' for biology¹ (spanning the years from 1514 to 1947), genetics² (1865–1955) and molecular genetics³ (1913–1963), not one was an assertion. But during the 1970s and 1980s the use of ASTs in biological journals has spread like an infectious agent.

Frequencies

The frequencies of ASTs were measured for biological sciences in samples from PNAS (*Proceedings of the National Academy of Sciences, U.S.A.*). Between 1960 and 1969 there were no ASTs out of a total of 2,582 titles examined. They first appeared in 1970 (3/429 titles or 0.7%), and by 1989 the frequency had risen to about 34%. The proportion of ASTs in *Cell*, a journal of molecular biology launched in 1974, paralleled that in PNAS until 1979. It increased sharply to 24% in 1980 and has been around 45% since 1986, the highest proportion for any journal examined.

In the figure, AST frequencies in 1982 and 1989 are compared for several other publications. Whereas such titles are infrequent in two chemistry journals, they are common in journals reporting on molecular biology. Given the wide variation from journal to journal, it would seem that editors have a large part in determining AST usage. But the figure

shows that molecular and cellular biologists tend to use ASTs much more often than do chemists; I found but one AST out of 378 (0.3%) articles categorized as 'chemistry' in PNAS from 1970 to 1979, compared to 100/3044 (3.2%) for 'biochemistry' over the same period. Furthermore



Frequencies of ASTs in ten journals in 1982 and in 1989. Several issues of the journal from a particular year were selected at random and then all of the titles listed in the contents were examined. Each sample consisted of 100 to 300 primary research articles. (JACS, *Journal of the American Chemical Society*; JCP, *Journal of Chemical Physics*; JMB, *Journal of Molecular Biology*; JB, *Journal of Bacteriology*; JBC, *Journal of Biological Chemistry*.)

ASTs in *Science* and *Nature* are largely in the biological sciences.

What led to the rise in use of these titles in the 1970s, and why are they mainly in molecular biology? I suspect that the answer to both questions lies in the publication in 1965 of James Watson's influential textbook, *The Molecular Biology of the Gene*⁴. Watson was an innovator in the use of ASTs as chapter titles and subheadings to stress that biology had come of age — no longer was there a need to beat about the bush; students could be told what is and what isn't known about molecular biology. Indeed, the heading of Chapter 2, "Cells Obey the Laws of Chemistry", boldly states the singular triumph of molecular biology, and lays to rest the bothersome, vitalistic notion that the rules for biology are different from those of chemistry and physics.

Watson's use of ASTs may have encouraged some scientists to adopt them, but why do they continue to rise in popularity? The answer, I believe, lies in the increasing pressure for science to be goal-orientated. On this view, the only acceptable outcome of a project is an 'answer', a product. And ASTs provide not only the product but the advertisement for it — they help the author to convince the journal, the reader and the granting agency

that the article is worth reading and that the work reached a successful conclusion. By advertising the paper in this way, the user of ASTs gains an advantage over the more traditional competitor who may be perceived as someone who looked but could not find. This, in turn, gives rise to 'title wars': a prospective author feels pressure to use ASTs just to keep up with the competition.

Merits

The merits of the traditional title are these — it states what the work is about in an open-ended tone, inviting the reader to judge the paper by its content not its title, and it reflects the author's appreciation of the possibility of error. In contrast, ASTs are improper and imprudent.

First, they are often patently unprovable (for example declarations that a process requires or does not require a certain component); only when all possible conditions have been tried can that be substantiated. Second, when a title turns out to be in error, the literature is left with that legacy. Even citing the 'good' parts of such a paper will be problematic, because (in many journals) the AST has to appear in the reference citation and valuable work may be buried under an erroneous title. Third, in some cases the AST boldly states a conclusion that is then stated more tentatively in the summary or elsewhere. Such practices misinform the reader who has come to rely on the title for the take-home message. Finally, ASTs trivialize a scientific report by reducing it to a one-liner. There is more to a paper than a single conclusion; the materials, the methods or the data may ultimately prove to be of more value than the advertised conclusion.

It is because ASTs declare science to be a product that they are to be deplored. By adhering to the idea of science as process — not product — we risk less and may ultimately achieve more. □

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Maxwell's demon flourishes

The perennial question of whether suitably instructed microscopic beings could falsify the second law of thermodynamics is still alive, but only just.

JAMES Clerk Maxwell's demon stands in relation to the second law of thermodynamics as does King Canute to the tides in the North Sea, between Britain and Scandinavia. Canute (995–1035), anxious to demonstrate to an over-trusting people that even he could not halt the diurnal tide, allowed his feet to be wetted uncomfortably by the sea. Maxwell, the founder of the kinetic theory of gases, was similarly concerned to show that the second law of thermodynamics could be avoided only by magical means: his demon was meant to be a sentient creature of atomic dimensions, able to discriminate between atoms and molecules.

If properly instructed, a small gang of Maxwell's demons could ensure that the entropy of an isolated system would spontaneously decrease. Take a volume of gas in equilibrium with itself, partition it into two by means of a solid screen and station a demon at an orifice in the screen. If the demon is instructed to prevent high-velocity molecules from moving in one direction through the orifice, and low-velocity molecules from moving in the other, the result will be that the temperature of one part of the accessible volume will increase and that of the other will decrease. Equilibrium will be upset.

Maxwell's argument resembles that of Canute in obvious ways. Canute demonstrated that he lacked supernatural powers. Maxwell showed that a microscopic sentient demon is the only way in which the consequences of the second law can be avoided and then appealed to the general knowledge that such creatures cannot exist. But neither Canute nor Maxwell would be entirely satisfied with the outcome. Belief in the supernatural flourishes, in various forms of parasceince. And there is a rich and flourishing "what if . . . ?" literature of Maxwell's demon which, by contrast, has the virtue of being interesting as well.

That is not surprising. There is, for example, the prior question of whether Maxwell's demon could function as described. Can a microscopic sentient being interfere with the motion of individual molecules so as to destroy thermodynamic equilibrium, and without the expenditure of effort? And then, because deviations from the second law would imply that strange phenomena — perpetual motion, for example — would be possible, could Maxwell's demon (if it exists) be harnessed to useful purposes?

Unsurprisingly, most people have concluded that there is not much scope for it. Carlton M. Caves, from the University of Southern California at Los Angeles, is the latest to have done so (*Phys. Rev. Lett.* **64**, 2111; 30 April 1990).

In principle, a demon should be able to do what is expected of it (him or her) without significant expenditure of mechanical effort, called work. Standing at the orifice in a partition, for example, the process of selecting between molecules of different characteristics would entail three consecutive steps — deciding whether or not an approaching molecule should be allowed to pass through the orifice, closing a frictionless door when the decision is to exclude it and then returning the system to its original condition in preparation for the arrival of the next particle. Making a strictly frictionless door would not, of course, be a simple matter, but there is no intrinsic lower limit to performance.

That is, of course, a recipe for perpetual motion; simply instruct the demon to maintain a constant difference of temperature between the two parts of the enclosed volume and use them as the upper and lower heat reservoirs of a heat engine.

Caves notes that the simplest engine of this kind is the *gedanken*-engine devised by Leo Szilard, in the century after Maxwell. Put a single molecule in an enclosure bounded by two frictionless pistons and equipped with a movable partition dividing the enclosure exactly into two. The partition is dropped, the demon decides in which half of the box the molecule is trapped, moves the piston closing the other half up to the partition (there is no resistance because that side is empty) and then removes the partition. The result is that the one-molecule gas does work on the piston of amount $kT \log_2 2$, where k is Boltzmann's constant and T the temperature. The quantity $k \log_2 2$ is simply the entropy of mixing between the two halves of the box. So is the second law false and, in particular, is perpetual motion possible?

Attempts to save the appearances by pleading that the demon must do some work have mostly been defeated by the argument that the friction of its equipment can be indefinitely reduced. But the demon's Achilles' heel turns out to be cognitive, not mechanical. A demon deciding that a molecule is in one half of a box and not the other must carry out a computation, store the result and then

clear its mind (or computer) for the next cycle, which entails the manipulation of entropy. Rolf Landauer (see *Nature* **335**, 779; 1988) is chiefly responsible for having made the case that the only unavoidable energy cost in the demon's work is that involved in clearing its mind for the next cycle of the operation. The intelligence that the molecule is on one side of the box rather than the other can evidently be represented by a single bit of information, but Landauer shows that the energy needed to erase it is precisely $kT \log_2 2$, so that the second law remains intact.

Caves goes a step further, by conceptually coupling together several (say N) Szilard *gedanken*-engines. He accepts Landauer's argument that the erasure of information is the superintending demon's most probable energy cost, but explores the question raised by W. H. Zurek (*Nature* **341**, 119; 1989) of whether it may be possible to avoid the stringency of the second law by collecting information economically, and thus having less of it to erase from the memory.

The trick lies in the briefing of the superintending demon, which must be told to extract work from the engine only when the storage of information can be handled economically. In the extreme case, for example, the demon might be told not to extract work except when the N molecules in the N containers are on the left-hand side of their respective partitions. Then, the work the demon can arrange to be produced is $NkT \log_2 2$, which state can be represented by only a single bit, the erasure of which will require only the expenditure of energy equivalent to $kT \log_2 2$. The argument, inevitably, proceeds by a combinatorial description of the states of the machine and a calculation of the shortest algorithm that will erase information of unpredicted complexity.

The outcome is teasing, but familiar. Caves concludes that Maxwell's demon could indeed extract work from a *gedanken*-engine, but only by waiting for thermodynamic fluctuations that are, by definition, rare. Designers of perpetual motion machines (an energetic company) will throw up their hands at this point, looking for something a little less chancy with which to impress their sponsors, but Caves seems to have hit on the foundation for yet another wave of speculation about what Maxwell's demon might accomplish.

John Maddox

Origin of organic materials

I. P. Wright and Iain Gilmour

THE results of a new investigation into the carbon isotope fractionation accompanying laboratory syntheses of organic materials¹ apparently contradict the findings of a previous study². The dispute was aired recently* when E. Anders (University of Chicago) replied to the work of S. Chang (NASA, Ames) and co-workers who have conducted these new experiments involving the Fischer-Tropsch-type (FTT) synthesis, the results of which may have important ramifications for understanding the origin of organic compounds in meteorites. The argument goes back some way with the protagonists taking opposite sides on the implications of such experiments. The central issue is whether the organic materials in meteorites were formed predominantly in the solar nebula or elsewhere — for instance, in a presolar environment or by secondary processes on primitive parent bodies.

Formation

To understand the conflict it is necessary to go back to the early 1950s when the processes responsible for the origin of organic materials on the Earth and in meteorites were first considered. It was H. C. Urey who reasoned that carbon dioxide in the Earth's primitive atmosphere could not transform spontaneously into organic compounds as these are thermodynamically less stable³. He proposed instead that if the atmosphere was composed of simple molecules of a reduced nature (that is, CH_4 , NH_3 , H_2O and H_2) then organic molecules could be formed if energy, in the form of ultraviolet light or electric discharges, was put into the system. This seemed reasonable enough and in 1953, in a classic experiment, S. L. Miller succeeded in producing amino acids (the precursors of life and, although not known at the time, also constituents of meteorites) by means of an electric spark discharge in a mixture of CH_4 , NH_3 and H_2O (ref. 4). At about the same time, it was noted by Urey⁵ that, in a gas of solar composition, the astronomically important CO molecule may have transformed into 'complex tarry substances' on cooling and compression. This has certain parallels with the Fischer-Tropsch synthesis whereby CO and H_2 are converted into organic materials using heated catalysts of various types.

During the 1960s and early 1970s, Anders and colleagues investigated the ability of the FTT synthesis to account for the nature and distribution of organic materials in carbonaceous chondrites. Twenty years ago, the prevailing hypothe-

sis was that the Solar System had formed from an initially hot, homogeneous gas of solar composition, so it is important to remember that Anders's experiments on FTT synthesis of organic materials were conceived and executed within this framework, and represented a natural extension of this hypothesis. From thermodynamic calculations it is known that the temperature of the nebula must have cooled to 300–400 K before various minerals, which are ideal catalysts for the FTT synthesis, could form. Some of these minerals (for example, hydrated minerals and magnetite) are known constituents of carbonaceous chondrites. Thus, some of the laboratory experiments used these various catalytic materials, along with H_2 and $\text{CO} \pm \text{NH}_3$ (a retrospective of the work is given in ref. 6). The reactions were carried out in a closed vessel, that is, under static conditions. The results are entirely convincing, the process giving rise, among other things, to aliphatic and aromatic hydrocarbons, alcohols, carboxylic acids and amino acids (all of which have been identified in carbonaceous chondrites). In addition, it was shown that the carbon isotope fractionation between the organic materials and the CO_2 formed during the reactions is analogous to the difference observed between the reduced and oxidized forms of carbon (organics and carbonates respectively) in carbonaceous chondrites. But it should be noted that the analytical capabilities of the time meant that the isotope fractionation experiments necessitated the use of a cobalt catalyst as it is more effective at lower temperatures (high yields were necessary in order to make the isotopic measurements) and native cobalt is probably not present in primitive meteorites. Nonetheless, the experiments provided strong evidence that an FTT synthesis was responsible for the formation of organic materials in the solar nebula.

Twenty years on, however, the solar nebula is envisaged to be much more complicated. Primitive meteorites abound with small amounts of peculiar components and isotopically anomalous materials which attest to the survival of presolar components. Indeed, at least part of the organic material in meteorites is apparently presolar with a high D/H ratio, thought to be a remnant signature of interstellar processing. In other words, parts of the solar nebula are not easily described in terms of the condensation products from a cooling solar gas — clearly a certain amount of presolar dust survived any heating events to be incorporated directly into the parent bodies of primitive meteorites. Furthermore, the isotopic

composition of meteoritic carbonates has now been tentatively explained as resulting from the incorporation of material derived from presolar grains⁷ such as silicon carbide which has a vast excess of ^{13}C .

Many independent lines of evidence show that meteoritic carbonates were formed by aqueous processes on primitive parent bodies. If this is true then perhaps organic materials were synthesized in this sort of environment as well. Indeed, it has recently been shown that the amino acids in carbonaceous chondrites could form from aqueous alteration of polycyclic aromatic hydrocarbons⁸, the latter thought to arise from interstellar grains, not from condensation processes in the nebula. Thus, the time is right for a reappraisal of the carbon isotope fractionation that apparently accompanies the FTT synthesis. And as Chang *et al.* have shown, there is now some cause to have reservations about the previously reported results. In the new experiments the FTT reactions were carried out in a dynamic, rather than a static, system. Furthermore, the catalysts now used reflect more faithfully what is present in carbonaceous chondrites (for example, hydrated silicates and magnetite). It was discovered that, under such conditions, no large carbon isotope fractionations were obtained between the oxidized and reduced products. It is clear that this is not due to the use of a different sort of catalyst because it is possible to reproduce the results of the previous study under conditions of high temperature or long duration, wherein most of the reactant CO is used up (in effect, equivalent to the conditions Anders had obtained at lower temperatures using a cobalt catalyst). In other words, the carbon isotope fractionation is not a consequence of the FTT synthesis but, rather, the result of having attained isotopic equilibrium. Interpretation of the meteoritic data, within the context of these new results, imposes a number of constraints on any putative nebula FTT synthesis (that is, temperatures must be as high as 500 °C and practically all of the CO in the nebula must be removed).

Fractionation

It is worth considering the relevance of a carbon isotope fractionation, or lack of one, during FTT synthesis. In the original experiments, the magnitude of the isotope fractionation was important primarily as a key piece of supporting evidence for FTT synthesis. Even if, as now appears to be the case, fractionation only results if the reaction goes to completion, it is important to realize that organic matter will still be produced in an FTT synthesis and the results from laboratory experiments broadly reflect what is present in meteorites. For instance, of the 10,000 possible isomers of C_{10} saturated hydro-

*Lunar and Planetary Science Conference XXI, Houston, Texas 12–16 March 1990.

carbons, only six are produced by laboratory FTT experiments, five of which are the dominant forms in meteorites. Any viable alternative synthesis experiments must reproduce this phenomenon. Unfortunately, few of the possible alternatives have received the detailed study necessary. The Miller-Urey synthesis does produce some matches, although not the C_{16} isomers, between the product mixture and meteoritic material, but was originally not accepted primarily because there was no isotope fractionation. Other photochemical processes have also been suggested for the formation of carbon-rich grains that might contribute to the organic matter of primitive Solar System materials. They involve the photoionization of reduced species, either in the gas phase⁹ or condensed on interstellar grains¹⁰. The products of such processes are, at present, poorly characterized but do yield macromolecular organic material, termed 'tholins'. But it is anticipated that, in general, the photochemistry of simple radicals will produce a structurally random mix of products and so may not match what is observed in carbonaceous chondrites.

With hindsight it is easy to appreciate that primitive meteorites consist of materials that formed at various times and places before the Solar System existed,

together with significant quantities of nebula products. It no longer seems necessary to explain the presence of organic materials in meteorites as the result of condensation processes in the solar nebula. Rather, the organics may largely be the result of FTT processes which occurred before the solar nebula formed¹¹. So it seems that the isotopic evidence obtained in the laboratory, once considered to support the notion that organic materials formed in the solar nebula by FTT processes, was misinterpreted. □

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SEXUAL DIFFERENTIATION

Of MIS and the mouse

Anne McLaren

TRANSGENIC mice are full of surprises. If one had even a modest understanding of the developmental genetics of a system, it should be possible to predict the consequences of over-expressing or under-expressing a gene, or expressing it in the wrong place or at the wrong time. This is seldom so. I doubt that Behringer and colleagues (reporting on page 167 of this issue¹) ever expected to produce transgenic female mice that totally lacked ovaries. How did this unexpected result come about?

Behringer *et al.* introduced into their mice the structural gene for a hormone identified by Alfred Jost, and termed by him the Müllerian inhibitor². Others since have called it anti-Müllerian hormone (AMH) or, as in the new report, Müllerian inhibiting substance (MIS). The early mammalian embryo has the wherewithal to develop in either the male or the female direction: both sets of reproductive ducts are provided in rudimentary form, and which set develops depends on whether the pair of gonads become testes (if a Y chromosome is present) or ovaries (if a Y is lacking). In a male embryo, MIS is one of the earliest products of the fetal testis, and causes the regression of the female

Müllerian duct system, so no oviducts or uterus are formed. Androgens produced by the fetal testis then stimulate the male Wolffian ducts to develop into epididymides, vasa deferentia and so forth.

Human and bovine MIS have now been purified and the genes cloned, but for years a bioassay based on the regression of the Müllerian duct in culture³ was the only method of detecting MIS. It is therefore not surprising that transgenic female mice expressing the human MIS gene under the control of the mouse metallothionein gene promoter should lack oviducts and uterus. Presumably MIS is expressed in the fetus at the time of sexual differentiation, although this has not been formally demonstrated.

The lack of ovaries in most of the adult transgenic females (those that have higher levels of circulating MIS) could by hindsight have been predicted. The addition of purified MIS to fetal rat ovaries in culture⁴ leads to a massive loss of germ cells, starting around the time of their entry into meiosis. Possibly one function of MIS in the fetal testis is to wipe out any oocytes that have entered meiosis despite the inhibiting effect of the testis cords. In the normal ovary, oocytes induce the support-

ing cell lineage to differentiate as follicle cells rather than as Sertoli cells, and it is the continued presence of oocytes that maintains follicles. When oocytes disappear, follicles no longer persist, and can be transformed into structures resembling testis cords⁵. When oocytes are present in the testis, the opposite transformation can occur, with Sertoli cells apparently induced to form follicles⁶.

Disappearance of oocytes, followed by occasional transformation of follicles into testis-cord-like structures, is also seen in the ovaries of female calf fetuses twinned with males, the so-called freemartin phenomenon. These effects too may be due to MIS, produced by the bull twin's testes and passed to the female by the common placental circulation.

The transgenic female mice produced by Behringer *et al.* have a reduced number of oocytes and follicles in their ovaries at birth; by two weeks no oocytes remain, but the authors report some structures resembling testis cords in the small gonads. In most of the adult females, no trace of gonads could be found. The authors suggest that the testis-cord-like structures may be induced by MIS, as they have not been seen in situations where germ cells are lacking from the start of ovarian development; but in these circumstances no follicles are formed, so perhaps the possibility of transformation into testis cords does not exist. The changes seen in the ovaries, and their ultimate disappearance, may all stem from the effects of MIS on the oocytes themselves.

There is, however, ample evidence that MIS has several functions in mammalian reproduction. It is produced by the follicle cells of the normal adult ovary, but we do not know what it does there. A few of the transgenic males have feminized external genitalia, abnormal Wolffian duct development, and undescended testes. The authors speculate that MIS is involved in the control of testicular descent, and that high levels inhibit the synthesis of androgens. Reproduction seems to make do with few hormones, with each used for many different functions: MIS is clearly no exception.

The next transgenic experiment must be to abolish the expression of MIS. Any guesses as to what the mice will be like? □

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Ancient crust on Pacific plate

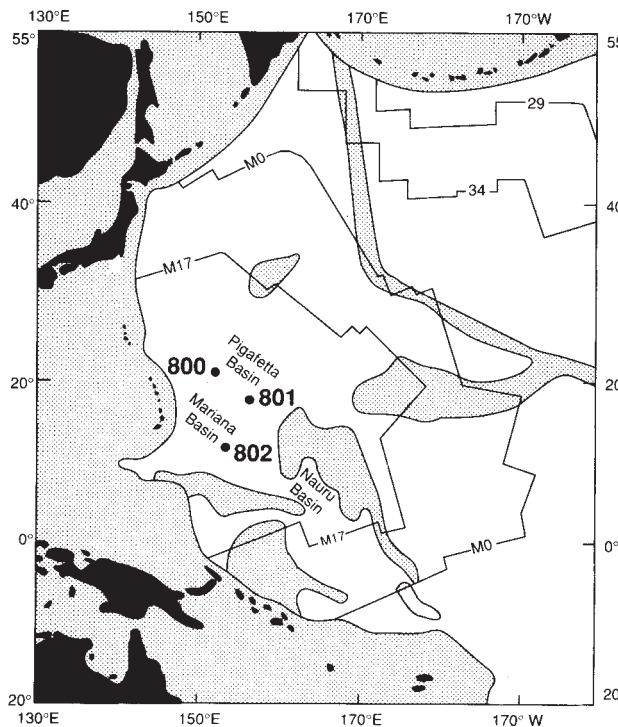
*Leg 129 shipboard scientific party**

AFTER a twenty-year search, oceanic crust and sediments dating from the Jurassic have been discovered beneath the deep western Pacific Ocean by Leg 129 of the Ocean Drilling Program (ODP). The oldest sediment recovered is about 170 million years old and is from the base of the Callovian Stage of the Middle Jurassic. This is equivalent to the oldest crustal section to have been recovered from beneath the world's oceans.

Mapping of magnetic anomalies in the western Pacific has revealed undated magnetic lineations, presumably of Mesozoic age (between 160 and 165 million years old), that might be the oldest magnetic anomalies remaining in any of the Earth's ocean basins. These lineations in the Pigafetta and East Mariana basins are bordered by magnetic 'quiet zones' which are potentially the oldest areas of the Pacific plate. It has been suggested² that an area equal in size to the continental United States or western Europe may be underlain by Jurassic sediments and oceanic crust (see figure), but no evidence of that has ever been recovered from the region, despite the attempts of eight scientific ocean drilling expeditions since 1969 and numerous dredging expeditions going back as far as the 1950s. Instead, the oldest material recovered has almost invariably been the remains of a huge, off-ridge volcanic event which blanketed the area with thick layers of dolerite sills, basaltic flows and volcanoclastic sediments in the early to mid-Cretaceous, between 80 and 120 million years ago.

Multichannel seismic reflection profiling throughout the deep basins of the western Pacific³ has revealed 'windows' through this Cretaceous volcanic pile, providing us with an opportunity for complete penetration into the supposed, original oceanic crust. With the help of

data from these seismic surveys, ODP sites 800, 801 and 802 were selected and subsequently drilled on Leg 129. Site 800 yielded only dolerite sills, and 802 basaltic lava flows, both of Cretaceous age. But at site 801, Jurassic sediments and extensive basement were uncovered after drilling through 200 metres of mid-Cretaceous



Coring site locations for ODP Leg 129 in the western Pacific Ocean. White portions of the map represent areas of normal ocean crustal thickness and water depth on the Pacific plate, and grey areas represent volcanic edifices with thickened crustal sections, as well as the younger areas beyond the Pacific subduction zones. Magnetic lineation isochrons² correspond to the following geological boundaries: M17, Jurassic/Cretaceous; M0-Barremian/Aptian; M34, Santonian/Campanian; and M29, Cretaceous/Tertiary.

(Albian) volcanoclastic turbidites.

The Upper Jurassic sedimentary section at site 801 contains marine fossils thought to be from the giant ocean that surrounded the supercontinent Pangaea at that time. The fossils are mainly radiolarians — plankton with a silica shell. The section comprises 70 metres of brown radiolarite together with an abundance of manganese oxide and dark brown chert. This material is underlain, probably unconformably, by 20 metres of red radiolarite and claystone of Callovian age which are heavily iron-enriched, giving them their colour. Biostratigraphic dating is based on a recently developed radiolarian stratigraphy⁴ for the Pacific. The basal sediments are believed to have been deposited during the Middle Jurassic near the Callovian/Bathonian boundary, but the oldest strati-

graphic age has yet to be confirmed by radiometric dating of the underlying basalts.

Magnetic remanence measurements and microfossil abundances suggest that the Pacific plate was formed north of the Equator during the Middle Jurassic; it moved south across the Equator at the end of the Jurassic and then started to go north in the mid-Cretaceous. Since then, it has moved northwards continuously to where it is now. It is significant that the fossils recovered are mainly composed of silica, as opposed to carbonate, showing that there was very little carbonate production and/or preservation in the Jurassic 'superocean'.

In holes 801B and 801C, we drilled through igneous basement to 131 metres below the interface between sediment and crust, with a 20-metre section of overlap between the two holes. The basement is made of interlayered lava flows and pillow basalts. From the physical appearance of the basalts, it seems that they were extruded mainly under submarine conditions and, from their location beneath Callovian/Bathonian sediments, that they were emplaced sometime during the Middle Jurassic.

Forty stratigraphic layers were recovered from the oceanic crust at site 801. Extrusive flows, mainly aphyric basalts, made up much of the material, although there were some microphyric and phyrlic basalts with olivine and plagioclase phenocrysts. We also recovered 3 metres of a hydrothermal deposit from about 60 to 70 metres below the top of the basement. The deposit probably consisted of iron oxides/hydroxides which have been well cemented, and largely replaced by silica. The pillow basalts beneath the deposit had been altered, presumably by the

action of the same hot-water solutions responsible for the hydrothermal deposit. This deposit is remarkable — it is particularly well preserved, probably because it became buried beneath extrusive flows soon after its deposition.

During Leg 129, no logging or other geophysical measurements were carried out in the basement part of hole 801C. But the hole has remained stable and clean, thus presenting a golden opportunity for further investigation of this section of oceanic crust. □

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Unusual atomic environments

D. M. P. Mingos

THE realization that the properties of molecules are determined not only by the types of chemical bonds present, but also by their three-dimensional arrangement in space, can be traced back to Pasteur and van't Hoff's research in the last century. Van't Hoff showed that the structure of the isomers observed in organic chemistry could be understood if it was assumed that the four bonds made by carbon were directed towards the vertices of a tetrahedron. This principle has become a central tenet of modern organic chemistry because there are so few exceptions to it. In recent years, however, inorganic chemists have discovered an increasing number of compounds where the carbon atoms and isoelectronic B⁻ and N⁺ ions are in unusual and non-tetrahedral environments. Indeed, on page 140 of this issue¹, Grohmann *et al.* (Schmidbaur's group) report the structure of a compound containing nitrogen in a very unusual coordination environment. Although nitrogen, like carbon, is limited generally to four tetrahedral bonds, in this case it has five gold atoms surrounding it.

Theoretical calculations have indicated that if the hydrogen atoms in hydrocarbons were replaced by lithium atoms then the resulting compounds could have exceptional three-dimensional geometries. However, molecules such as CLi₆ are very difficult to make because of their extreme sensitivity to moisture, and so these possibilities have not been seriously explored by the majority of organic chemists.

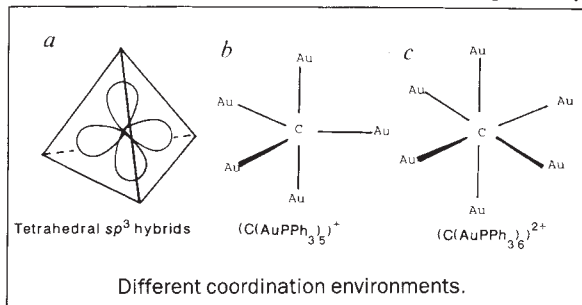
During the 1970s an increasing number of cluster compounds with carbon in unusual coordination environments were discovered. In these compounds, transition metals, which are less susceptible to moisture than lithium, are bonded together in much the same manner as in the parent metal, but contain only a finite number of metal atoms (up to 50) whose surface is coated with a protective layer of molecules such as carbon monoxide. In these clusters, cavities are generated at their centres which are large enough to accommodate carbon and other main group atoms, for example nitrogen, boron, sulphur and phosphorus. The carbon atoms in these clusters can be surrounded by up to eight metal atoms, and in geometrical environments more familiar to mathematicians than organic chemists: octahedral, square-pyramidal, trigonal prismatic and square antiprismatic. The bonding in these molecules cannot be described by localized tetrahedral *sp*³ hybrid orbitals conventionally used in organic chemistry (see figure *a*) and so it is necessary to adopt a delocal-

ized molecular orbital bonding description in order to account for their electronic and stereochemical requirements.

It has been argued that these unusual coordination environments for carbon are not relevant to organic stereochemistries, because the bonding situation is more closely related to that found in metal carbides, for example tungsten carbide, with infinite lattice structures rather than molecular organic compounds. Previously Schmidbaur *et al.*^{2,3}, however, demonstrated that it is possible to find carbon in unusual coordination environments even when the metal-metal bonding is not particularly strong. In particular, they showed that compounds where the carbon is bonded to gold are particularly effective at bringing out high coordination numbers for carbon. The Au(PPh₃) fragment (where

Ph = phenyl, C₆H₅) has a bonding capability which is similar to that of hydrogen and lithium atoms, because it uses only a single orbital for chemical bonding and this orbital is occupied by one electron. Inorganic chemists describe this similarity between H, Li and Au(PPh₃) by the term isolobal, suggesting that the groups have similar 'orbital lobes' for bonding.

Schmidbaur *et al.*^{2,3} made and characterized [C(AuPPh₃)₅]⁺ and [C(AuPPh₃)₆]²⁺ as shown in figures *b* and *c* respectively,



which are formally related to CH₄⁺, CLi₅⁺, CH₆²⁺ and CLi₆²⁺. In *b* and *c* the gold atoms are arranged in trigonal bipyramidal and octahedral environments respectively. Although they do not violate the inert gas

Reflections on mating behaviour

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

By use of the large mirrors shown here, lesser flamingos at The Wildfowl and Wetlands Trust, Slimbridge, Gloucestershire, are being duped into the belief that a small flock of them is much larger. Thereby, it is hoped, they will be encouraged to breed. The mirrors were installed earlier this year, and the success (or otherwise) of the ruse will emerge in June when the birds are due to lay their eggs.

In the wild, in Africa, lesser flamingos occur in huge flocks and engage in mass displays before breeding. The question is whether the presence of large numbers of birds is necessary for nature to take its course.

The flock at Slimbridge numbers 30. The birds have been through a period of display which (as in the wild) is now over, and the birds all seem to be paired up. They last laid eggs in 1984, and next month it will become known if the six-year barren period is over and if 60 will do when 30 did not.

Tim Lincoln

(or octet) rule because there are only eight valence electrons around carbon, their geometries cannot be explained in terms of the tetrahedral sp^3 hybrid orbitals shown in figure *a*. The distances between the gold atoms in the complex are much longer than those in the parent metal and so these compounds cannot be dismissed simply as interstitial metal carbides.

Grohmann *et al.*¹ now demonstrate that the same gold-phosphine unit can also create unusual coordination environments for nitrogen. The ammonium ion, NH_4^+ , is isoelectronic with CH_4 and is also tetrahedral, but the corresponding ions NH_5^{2+} and NH_6^{3+} are unknown. The authors find that the isolobal gold compound $[N(AuPPh_3)_5]^{2+}$ has a trigonal bipyramidal geometry (similar to that in *b*) and briefly mention that another German group have made $[N(AuPPh_3)_6]^{3+}$ which is analogous to structure *c*.

The compound $[C(AuPPh_3)_6]^{2+}$ is very closely related to $[Au(P(tol)_3)_6]^{2+}$ (*tol* = para-tolyl, 4-Me- C_6H_4), which was formulated as an octahedral cluster without an interstitial carbon atom in 1973⁴. But molecular orbital calculations on this cluster⁵ indicated that such a formulation was unlikely because it violated the octet rule. In $[Au_6(P(tol)_3)_6]^{2+}$ there are only four valence electrons involved in bonding because each $AuP(tol)_3$ fragment contributes one electron and the cluster carries a total charge of 2+. I proposed in 1976 that if this compound were reformulated as $[C(Au(P(tol)_3)_6)]^{2+}$ the additional four electrons donated by the carbon would make it conform with the requirements of the octet rule. The work of Schmidbaur's group has realized this theoretical prediction and confirmed that $[Au_6(P(tol)_3)_6]^{2+}$ is more correctly formulated as $[C(Au(P(tol)_3)_6)]^{2+}$. So, although these compounds do not conform to the rigid principle that all carbon atoms must have four tetrahedral bonds, their formulae can easily be predicted within a more delocalized description of the bonding derived from molecular orbital theory. New theoretical calculations⁶ have defined the closed shell requirements of analogous compounds with two carbon atoms at their centre. It will be interesting to see whether these predictions turn out to be as accurate as the previous ones. □

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Taking a cool look at iron

Anthony G. Davies

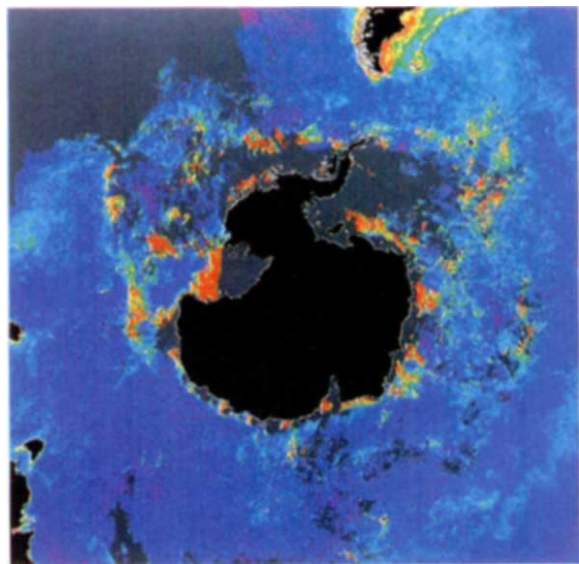
THE rate of photosynthetic carbon fixation by marine phytoplankton is normally controlled by light, temperature and the 'fertilizers' nitrate, phosphate and (for diatoms) silicate. But results of a study by Martin *et al.*, reported on page 156 of this issue¹, suggest that in the open Antarctic Ocean photosynthesis is restricted by a shortage of iron. Iron is a particularly important micronutrient because it is instrumental in the biosynthesis of chlorophyll, and is a main component of ferredoxin which facilitates the intracellular transfer and storage of photosynthetically incorporated energy. Martin *et al.* speculate that, in glacial periods of the Earth's history, iron transported to the Antarctic in wind-borne dust from other continents may have alleviated the iron deficiency. This, they argue, could have stimulated phytoplankton growth thereby increasing the amount of carbon dioxide drawn into the ocean from the atmosphere and contributing to global cooling.

The Antarctic Ocean is unusually rich in nutrients. Concentrations of nitrate, phosphate and silicate are 15–30, 1–2 and 40–90 $\mu\text{mol per kg sea water}$, respectively² (up to three times the corresponding maximum concentrations in the Atlantic water entering the English Channel). On the other hand, chlorophyll *a* concentrations (measures of the phytoplankton biomass) are low (0.1–1 $\mu\text{g per kg sea water}$): with a conservative estimate of 1 g chlorophyll *a* per g atom nitrogen in phytoplankton³, consumption of the nitrate in a bloom would be expected to produce at least 15 $\mu\text{g chlorophyll a per kg sea water}$. Similarly, carbon fixation — typically 0.1 g carbon $\text{m}^{-2} \text{d}^{-1}$ (ref. 3) — is as slow as that in severely nutrient-depleted oligotrophic regions of the Earth's oceans.

It was suggested as long ago as 1942 that the high productivity of the coastal areas relative to the open Antarctic Ocean is due to the presence of iron or manganese derived from the land⁴. Most of the subsequent attempts to demonstrate that a shortage of trace elements limits phytoplankton productivity have been inconclusive, mainly because of problems in obtaining uncontaminated samples of sea water. When anticontamination techniques have been used, some metal concentrations in the oceans have been shown

to be less than previously believed⁶. In particular, for the Gulf of Alaska, another nutrient-rich region, iron concentrations are low enough to limit phytoplankton growth^{7,8}. With their new study, Martin *et al.* now extend these results to the Antarctic Ocean.

Iron in sea water is present mainly in colloidal-sized particles of aged hydrous ferric oxide, essentially finely dispersed rust. On the basis of its solubility, the concentration of iron in true solution



False colour satellite image of Antarctic Ocean's summer phytoplankton distribution (red, most dense; blue, least dense).

should be less than 0.1 nmol per kg sea water⁹. Martin *et al.* find that 'dissolved iron' concentrations for the Gulf of Alaska⁸ and the open Antarctic Ocean¹ are mostly higher, especially in the deep water. Here the authors use the term 'dissolved' to describe the fraction of iron that passes through 0.4- μm pores of a polycarbonate membrane filter. Whether this iron is truly in solution is central to the debate because there is uncertainty over whether phytoplankton can assimilate iron directly from particles. Although some species produce siderophores — chelating substances with high affinities for iron — to mobilize iron from particles¹⁰ adhering to cell surfaces, it is believed that phytoplankton incorporate only soluble iron produced by the dissolution of the particulate form¹¹.

Martin *et al.* give no information on total particulate iron in the Antarctic Ocean. In the Gulf of Alaska, concentrations of iron up to 2.4 nmol per kg sea water were present in the top 100 m (ref. 8). Most of this was refractory (unleachable even by 25 per cent acetic acid), so it is not a large source of iron for phytoplankton. The atmosphere therefore

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probably supplies the iron necessary for maintaining the little growth actually occurring^{7,8}. It is not clear, though, why the biological availability of aeolian iron settling on the ocean should be any greater than that already in it. In any case, the remoteness of the Antarctic Ocean from the continents results in very low deposition rates, unless, as postulated for the glacial periods, unusual weather conditions prevail⁷.

Martin *et al.* hint at the possibility of counteracting global warming by using iron fertilization of the Antarctic Ocean to stimulate phytoplankton growth and thus increase the removal of carbon dioxide from the atmosphere. Assuming (1) that the volume of illuminated nutrient-rich water in the Antarctic Ocean is 3.2 million km³, (2) that the nitrate concentration is 25 µmol per kg sea water, and (3) the Redfield value of 6.6 for the carbon to nitrogen atomic ratio in phytoplankton, use of all the nitrate should draw down 6.4 × 10⁹ tonnes of carbon (as carbon dioxide) from the atmosphere. This is roughly the same as the annual input of carbon to the atmosphere as a result of fossil-fuel consumption and deforestation¹². Much of the fixed carbon would initially be carried down into deeper water in moribund phytoplankton and zooplankton faeces.

ASTROPHYSICS

Astronomical jet engines

Philip Hughes

OBSERVATIONS made over the past two decades have shown that a remarkable number of astronomical objects display jets — apparently well-collimated (beam-like) outflows of mass and energy^{1–3}. Such flows are associated with active galaxies (and perhaps our own Galaxy), young stars and stellar remnants such as neutron stars. They present the theorist with many challenges, not least of which is to explain their initial collimation. The great majority of current models invoke a disk or torus, tied gravitationally to some massive body, and the collimation is a consequence of a 'funnelling' of material along the system's rotation axis — either by the disk material, or an associated magnetic field. In a novel approach to this problem described on page 136 of this issue, Bell⁴ suggests that collimation might be achieved by the anisotropic propagation of radiation through the environment of a 'central engine'.

In early models⁵, jets resulted from the formation of bubbles of hot plasma, each with a radius of tens of light years, breaking out of an active galactic nucleus along 'the path of least resistance', namely, the rotation axis of the system. But at that time radio interferometers such as the Very Large Array (VLA) and the Multi-Element Radio-Linked Interferometer

There, decomposition of the organic matter and movement of the deep Antarctic water northwards to warmer areas would eventually return the carbon dioxide to the atmosphere. The effectiveness of such a project would clearly depend on the time-scales of these processes — not to mention a method for uniformly dispersing at least a million tonnes of iron in a usable form. □

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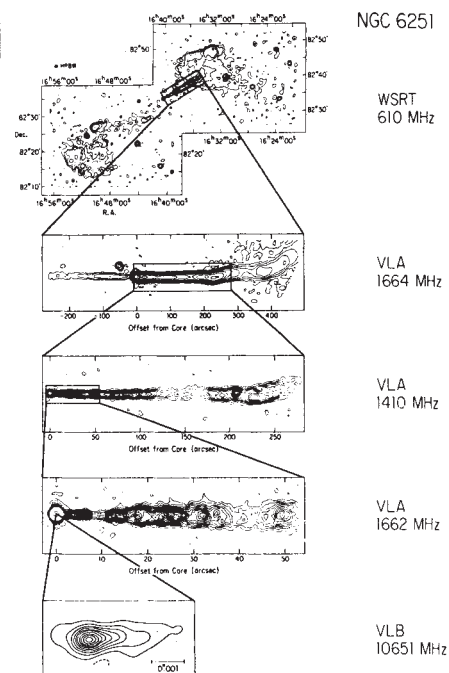


FIG. 1 Maps of the galaxy NGC6251 showing contours of radio brightness at a range of linear scales¹. The structure in the top panel spans several million light years — much larger than the associated optical galaxy; (whereas the lowest panel covers only a few light years). The 'jet' may be traced continuously from intergalactic to nuclear scales. (Courtesy of A.H. Bridle¹.)

electrons despite the low density — if coupling occurs, the ions' energy can be transferred to the electrons, radiated away, and the disk will not inflate. If the mass-accretion rate is high, the disk density will be high, and the disk will radiate strongly. But in this case, the radiation pressure will cause a bloating of the structure and so a 'radiation-pressure-supported torus' will form. It has become clear that such tori may be unstable⁷ and it is quite possible that the same will be true for ion-pressure-supported tori.

To compensate for the uncertainty over the stability of different structures, theorists have constructed models for the generation and collimation of energy for all of the recognized classes of disk structure. For geometrically thin disks, collimation can be achieved through the action of a large-scale magnetic field threading the disk, or through the influence of a wind from the upper and lower surfaces of the structure. Particles trapped in the 'funnel' of a radiation-pressure-supported torus are accelerated by the outwardly directed radiation pressure (although the effectiveness of this process seems to be severely limited). In contrast, the inner edge of an ion-pressure-supported torus can sustain electric currents whose associated magnetic field can thread the event horizon of the putative central black hole of an active galaxy and extract its rotational energy (see Fig. 2).

Disks (thick or thin) are natural candidates for the energy-generating and colli-

Network (MERLIN) had yet to provide the astronomical community with the images (see Fig. 1) that now form the observational core of the field, and inter-continental baseline interferometry had yet to reveal a direct link between intergalactic-scale jets and their associated galactic nuclei. With these advances came the realization that extragalactic jets must originate on scales much smaller than previously imagined.

It has long been realized that the accretion of matter by any object must involve the formation of an accretion disk or torus. Infalling material will possess significant angular momentum, which must be transported away from the inflow by viscous or magnetic stresses in the disk. Although many models assume that the disks are geometrically thin, general arguments suggest that they will become 'bloated'⁶; if the mass accretion rate is low, the disk density will be low, and the disk might not be able to radiate away the energy released by the stresses that enable the transport of angular momentum. The result is a high internal ion pressure which gives rise to an 'ion-pressure-supported torus'. But the survival of such a structure depends critically on whether or not some plasma process can couple the ions and

mating engines* of extragalactic and galactic jets. Energy release is from the inner edge of the accretion structure, or even from its anchoring central mass, thus satisfying the requirement that the jet has to be on a very small scale. The disk defines an

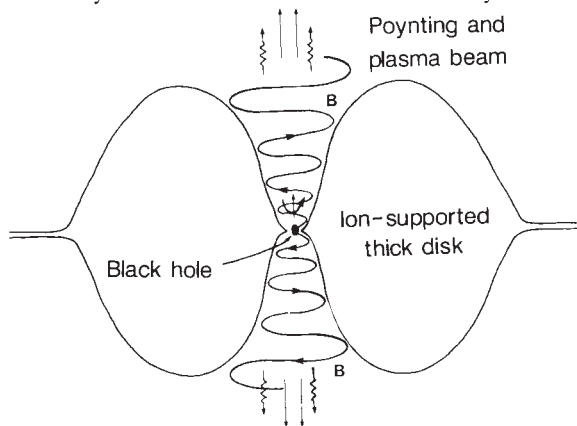


FIG. 2 Accretion disk around a black hole. The origin of the plasma jet or beam is thought to be connected in some way with the accretion structure. (Courtesy of P. J. Wiita⁹.)

axis of symmetry and this, together with the ubiquity of disks (presumably wherever accretion occurs), can explain the common occurrence of astrophysical objects that appear to have both a jet and counterjet. Furthermore, although the process of jet production remains unobserved, there is strong evidence for the presence of accretion structures: for example, Doppler-shifted emission from masing cloudlets which implies that they are embedded in a rotating structure associated with a galactic bipolar molecular flow; and thermal ultraviolet radiation from active galactic nuclei (AGNs) — plausibly explained as coming from a radiation-supported torus surrounding a black hole. Disks also provide a very promising framework in which to understand the various classes of AGNs. For example, it has been argued that radio-quiet quasars contain radiation-supported tori in which the radiation field has choked the funnel thereby inhibiting a jet, whereas radio galaxies house ion-supported tori wherein most of the energy release occurs as a radio jet, with little higher-frequency radiation arising from the torus itself.

Given the uncertainty over what disk structures are viable, it would be appealing to have a mechanism for jet formation that can ensure some degree of flow collimation for a broad range of geometries and physical conditions. Bell's work provides the framework for such a model by drawing attention to the fact that energy propagating in the form of electromagnetic waves is subject to inverse synchrotron absorption, which is a very strong function of the direction of the local magnetic field. Any large-scale magnetic field structure with the sort of geometry that one would associate with a dipole field, for example, will lead to anisotropic absorption, and hence to a 'photosphere' (optical depth of

order unity) whose surface near to the field's axis forms a narrow channel about that axis. Depending on the distribution of field and matter, the photosphere can lie outside the physical boundary of the system in the equatorial plane, in which case the emission will be collimated along the axis of symmetry. The model depends on the maintenance of a large-scale magnetic field, but this is also an attribute of the thin-disk and ion-pressure-supported-tori models. The great strength of Bell's model is that it does not depend on the details of the local field and matter distribution. As it hinges only on their large-scale distribution, it is able to accommodate both a broad range of geometries and disturbances, which could include instabilities in the accreting material.

To form plasma jets, the collimated electromagnetic wave energy must be transferred to 'blobs' of plasma. Nevertheless, and particularly for observers near to the symmetry axis, some or much of the original, driving, radiation field may be observable. This might provide an

MUSCLE REGULATION

On the other hand . . .

Ed Morris

IN CARDIAC and skeletal muscles the regulation of contraction is mediated by two proteins, troponin and tropomyosin, which are associated with the thin (actin) filaments (see Fig. 1). Troponin is composed of three polypeptide chains which can be separated and show characteristic activities. Alone among the troponins, troponin C has been crystallized and its atomic structure solved to a resolution of 2.8 Ångströms¹. Two papers on pages 132 and 182 of this issue^{2,3} illustrate the value of using site-directed mutagenesis and the knowledge of the atomic structure of troponin C in designing experiments to probe the mechanism of the regulatory process.

Within the troponin C crystal, the molecules are extended structures, each with two domains connected by a single α -helical strand (Fig. 2). Each domain has two Ca^{2+} -binding sites: the carboxy-terminal domain, containing sites III and IV, which bind Ca^{2+} or Mg^{2+} and are termed the high-affinity sites; and the amino-terminal domain, containing the low-affinity sites I

and II, specific to Ca^{2+} , and believed to be the regulatory sites. Each of the Ca^{2+} -binding sites is made up of a helix-loop-helix motif in which the Ca^{2+} is bound in the loop region: this motif is observed in a wide range of Ca^{2+} -binding proteins. The α -helical segments in troponin C are labelled A-F. Each Ca^{2+} -binding site is flanked by a pair of these α -helical segments — site I is flanked by segments A and B, and so on. The form

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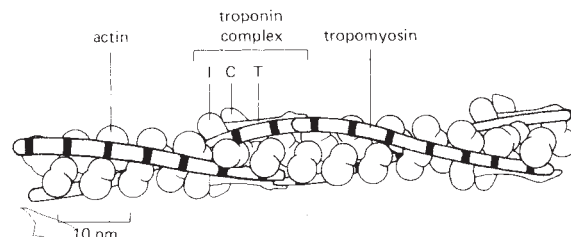


FIG. 1 Muscle thin filament showing the position of tropomyosin and the troponin complex on actin. The tail of tropomyosin T binds to tropomyosin. Troponin I has the ability to bind to actin and inhibit actomyosin ATPase and force production. The inhibitory action of troponin I is modulated by troponin C: on Ca^{2+} binding to troponin C, the inhibition is reversed. From *Molecular Biology of the Cell*.

in which the molecule was crystallized probably corresponds to the conformation associated with the relaxed state of muscle: Ca^{2+} binds to sites III and IV but

sites I and II are empty. A comparison of the conformation of the two types of site in the crystal structure led Herzberg *et al.*⁴ to propose that Ca^{2+} -binding to sites I and II causes a transition to a conformation more closely resembling that seen in

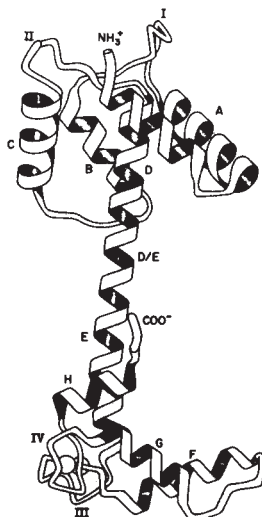


FIG. 2 The fold of the troponin C crystal. The upper helical domain is the calcium regulatory domain; the lower is the high-affinity calcium-magnesium binding domain (from ref. 1).

sites III and IV. According to this model, binding of Ca^{2+} is accompanied by a rearrangement of the α -helical segments on each side of each Ca^{2+} -binding loop so that they are less close to being anti-parallel and diverge more sharply.

The authors of the two papers in this issue seek to test this model by using genetic-engineering techniques to restrain the rearrangement of the helical segments and observing the effects upon the activity of the modified troponin-C molecule. Both sets of authors introduced some type of link between α -helical segments C and D. Fujimori *et al.*³ make use of the carboxylate-carboxylate interaction observed in the crystal studies between glutamate 57 and glutamate 88 on the C and D α -helical segments, respectively. The interaction is only favourable at the low pH used for crystallization where Ca^{2+} -binding to sites I and II is not observed. But if one of these glutamate residues is replaced by lysine then a salt-bridge might be formed which would be stable at physiological pH. Fujimori *et al.* have made both of these replacements. In each case they observe a reduction in the Ca^{2+} affinity of sites I and II. They also tested the regulatory ability of the mutated troponin C molecules by introducing them into skinned muscle fibres and observing the tension response to Ca^{2+} . In each case the tension increases only at higher Ca^{2+} concentrations.

By contrast to the ionic link used by Fujimori *et al.*, Grabarek *et al.*² introduced a covalent disulphide link, in this case between cysteine residues

introduced at positions previously occupied by glutamine 48 and glutamine 82 on the C and D α -helical segments. Thus direct evidence for the disulphide link can be readily obtained and the disulphide bond can be reduced to provide a good control against which to test the effects of crosslinking the α -helices. In agreement with Fujimori *et al.*, Grabarek *et al.* observe a reduction in Ca^{2+} affinity of sites I and II. Grabarek *et al.* also tested the regulatory function in two ways. First, they looked at the interaction between troponin C and troponin I. Regulation of contraction by troponin involves the Ca^{2+} -dependent modification by troponin C of the inhibitory activity of troponin I by allowing a more extensive interaction between the two proteins when Ca^{2+} is bound to sites I and II of troponin C. In agreement with this hypothesis, the uncrosslinked troponin C shows an enhanced affinity (about 50 $\times$ increase in affinity constant) for troponin I upon binding Ca^{2+} to sites I and II. The affinity of the crosslinked form for troponin I is enhanced less (about 10 $\times$) when Ca^{2+} is bound. Second, they looked at the regulation by Ca^{2+} of ATPase in myofibrils in which the cross-linked mutant troponin C shows little activation (about 20 per cent) of the ATPase upon Ca^{2+} binding, in contrast to the uncrosslinked form (about 90 per cent).

Thus, both studies reported in this issue support the conformational change in troponin C associated with Ca^{2+} -binding to sites I and II proposed by Herzberg *et al.*⁴. But complete transition to the proposed Ca^{2+} -bound conformation seems not to be a necessary conse-

quence of Ca^{2+} -binding, otherwise the Ca^{2+} affinity of sites I and II in the mutants with linked helices would be abolished rather than reduced. Conversely, linking the α -helices seems to stabilize the 'off' state of the troponin complex so that, even when Ca^{2+} is bound, transition to the 'on' state, as judged by the regulatory effect of the protein, is incomplete. So a type of experiment principally designed to test the conformational effect of Ca^{2+} binding also provides information about the next step in the regulatory pathway — the interaction with troponin I.

In providing a basis for the design of these experiments, the model of Herzberg *et al.* has served the authors well. But is it correct? One could envisage other structures for the 'on' state which would be consistent with the present results. The most satisfactory way to answer the question would be to solve the structure of troponin C in which calcium is bound to sites I and II. As there is no immediate prospect of this being achieved, another approach might be to trap the 'on' state using the type of experiment described by Fujimori *et al.* and Grabarek *et al.* □

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ARCHAEOLOGY

Made in the New World

Paul G. Bahn

DISCOVERIES by a French team at Puerto Chacho in northwest Colombia have pushed back estimates of the date of the appearance of pottery in the New World to the fourth millennium BC (ref. 1). They may serve finally to knock on the head the diffusionist theory that the New World received its first ceramics from Asia — pottery from the Pacific coast of Ecuador, at sites dating to the early third millennium, was previously thought by some² to show links with Japan, where the earliest pottery known dates to at least 12,000 years ago.

The often heated debate between the proponents of diffusion and of independent invention centred on the similarity of some sherds from Valdivia, Ecuador (third millennium) to fragments of early to middle Jomon pottery from sites on the Japanese island of Kyushu, dating to roughly the same period. Both were thick-

walled wares built from coils, with a smooth polished surface and a variety of incised decorations. Diffusionists argued that the stylistic similarities showed that pottery-making was introduced to Ecuador (and hence to the New World as a whole) by Japanese fishermen accidentally carried across the Pacific by storms and currents. The fact that no local antecedents were known for the Valdivia ware reinforced this impression of a sudden introduction from outside.

Critics, however, pointed to the immense distance involved (15,000 km), and to the fact that only small dug-out canoes are known from Jomon Japan, which could not have undertaken such a drift voyage³. They suggested that the similarities between the two wares reflect the limited possible range of variation in incised designs, and result from accidental convergence rather than culture contact.

This case was strengthened when earlier, more primitive pottery (San Pedro ware) was discovered stratified beneath Valdivia pottery at Valdivia itself⁴; although this also had incised decoration, it bore little resemblance to Jomon motifs. Yet even San Pedro pottery was still too sophisticated to be a first step in ceramic produc-

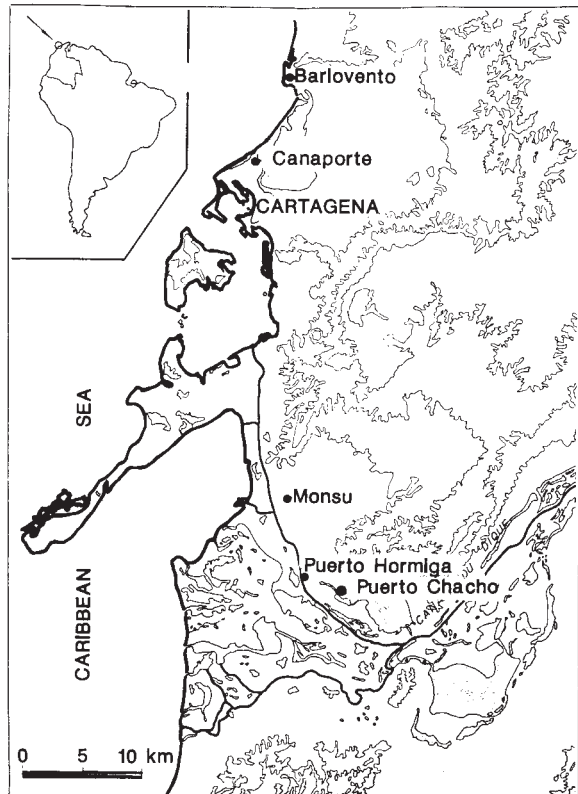
of the inhabitants' food came, inevitably, from the nearby aquatic resources: primarily oysters and limpets from the mangrove. A large type of shell, *Strombus gigas*, did come from the sea, and was used to make utensils. A preliminary study of fish remains shows a high degree of human selection among the many species available:

two marine species alone (*Centropomus* and *Eugerres*), which come up-river during spawning, account for over 50 per cent of the remains. The inhabitants fished mainly in the river, systematically exploiting the available resources according to season: they seem to have camped at Puerto Chacho in late autumn. In addition, a few other animals (turtle, cayman, iguana, manatee) were taken from time to time, as well as the occasional deer. As yet, no trace of agriculture or horticulture has been detected at the site, but this is hardly surprising given that it was occupied seasonally.

The early pottery's forms are simple, mostly hemispherical, and resemble the ware of Puerto Hormiga in the frequent use of plant fibres as tempers. Decoration includes incised lines, straight or curved, forming complex but highly organized motifs; dotted ornamentation; and zoomorphic handles, mostly depicting birds. Many of the decorated vessels still bear traces of a red colouring material on their surface. Although fired at low temperature, the pottery reveals a mastery of pyrotechnology which, together with the sophistication and variety of decoration, strongly supports the idea that ceramic production was already well established, and hence that its origins must lie further back in the fourth millennium, if not before.

The finds at Puerto Chacho have no clear links with the Ecuador material. They suggest that the inhabitants of America, like those of other parts of the world, may have invented pottery quite independently, and possibly in more than one area. □

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Map of northwest Colombia showing main archaeological sites discussed in the text.

tion, so the problem of its origins remained. The most likely source appeared to be Colombia.

The first archaeological work of note in the alluvial plain just south of Cartagena near the Caribbean coast (see map) was carried out in the 1960s⁵. Puerto Hormiga, for example, was a camp of fisher-gatherers of the early third millennium BC. The nearby site of Monsú⁶ was earlier, but its pottery was much the same as at Puerto Hormiga, and both were similar in date and style to the San Pedro ware.

The French team¹ began work in the region in 1987, and recorded about 30 sites. Puerto Chacho, close to Puerto Hormiga, is an elongated shell mound, 100 m long, 30 m wide and up to 1.2 m deep, located by the ancient terrace of a tributary and a few metres above a mangrove. Its stratigraphy comprises four main levels: layer 2b in the second level, containing pottery, has produced an uncalibrated radiocarbon date of 3270 ± 90 years BC.

Puerto Chacho seems to have been both a habitation and a refuse dump. At the periphery, where shells are absent, there are traces of a simple dwelling structure from the site's final occupation. Most

Seven-metre boots

OUR upright stance and walking gait have evolved very recently, and are still quite imperfect. In particular, we are designed to plod slowly through bush and rough country, and our legs are seriously undergeared. Evolution has still not reacted to that crucial invention, the smooth road. With the aid of a bicycle, we can propel ourselves at up to ten times normal walking pace on such a road. Daedalus is now devising a simpler way of matching us to our terrain. Inspired by the humble roller-skate, his new 'active boots' gear up the human stride to match any type of surface.

These cunning boots intercept the energy of each stride and use it to drive small wheels under their soles via a variable gearbox. At each stride, therefore, the wearer travels an additional distance provided by wheel rotation. The highest gear-ratio, appropriate for very smooth, level floors and roads, sends the wearer scudding a full ten stride-lengths for each stride he takes, speeding him along at bicycle velocity. Lower gears give less speed but more power, for rougher terrain like carpet or uneven tarmac. The lowest gear of all, for really rough ground or obstacles like stairs, gives zero gain. It locks the wheels, converting the boots back to normal passive footwear.

DREADCO engineers are wrestling to implement this ingenious concept. Their prototype captures stride-energy very simply, by means of a cord connecting the two boots. As the wearer strides along, the cord unwinds and rewinds from a tiny windlass in each boot; a spring-and-ratchet mechanism stores the captured energy and feeds it to the wheels via a continuously variable automatic gearbox. This sets its ratio from the tension in the cord, thus matching the wearer's muscular effort to the resistance being felt by the wheels. A free-wheel system lets the wearer 'coast' between strides, saving energy and preventing sudden changes of velocity which might tip him over.

The final design should be so well adapted to human walking and balancing reflexes as to be hardly noticeable. The wearer of DREADCO's 'Hyperboots'[®] will have no sense of pedalling a mechanism; he will just find himself speeding along as if walking on a moving conveyor-belt. Life will be utterly changed. The postman, the shopwalker, the canvasser, even the housewife and laboratory technician who walk unnoticed miles in the course of a day will all feel a wonderful new ease and freedom. Hyperboots will magically shrink all distances, indoors and outside, wonderfully increasing the 'strolling range' we can traverse without vehicular assistance. Exciting new sports, from Hyperboot racing to high-speed dancing, should also emerge.

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Helpline for giant clams

SIR—Hundreds, possibly thousands, of giant clams (*Tridacna* sp.) are being cultured commercially in the Caribbean, possibly without any screening for diseases. To our knowledge, more than 600 specimens are being cultured in Bonaire, others in Guadeloupe and in south Florida, and there are unconfirmed reports of other projects in the Caribbean and of planned exhibitions in north Florida and Arizona. The Florida and Bonaire clams are from Palau, Micronesia. We do not know if any of these animals have been tested for pathogens.

We would like to get in touch with all those rearing giant clams in the Atlantic so that we can arrange proper disease screening. There is an urgent need to avoid introducing pathogens which may harm the conduct and reputation of aquaculture or damage Caribbean fisheries. In advocating testing, we do not condone the introduction of *Tridacna* sp. outside their previous natural ranges, but take a practical approach in an attempt to minimize possible damage.

Several projects in the Pacific are attempting to rear *Tridacna* sp. for their economically valuable adductor muscle, mantle tissue and shells¹. However desirable giant clams may be, their diseases, once introduced, may not be easy to eliminate: pathogens accompanying introduced organisms represent an uncontrollable experiment. The most serious pathogen known to occur in *Tridacna* sp. is a *Perkinsus* species, *api-complexan*, which has caused mass mortal-

ities^{2,3}, and which, alarmingly, seems to possess little host specificity². This genus causes death and disease in bivalves world-wide^{2,4}. Guidelines to prevent the transfer of various microbial associates have been accepted by some of those who distribute and culture these clams in the Pacific (C. E. Birkeland and L. G. Eldredge, personal communication).

Our Caribbean Aquatic Animal Health Project (telephone number 809 899 2048) does not have the authority to prohibit introduction or to certify disease-free stocks of *Tridacna* sp., but we do seek to establish cooperation between those who are holding these animals in the Atlantic and those working on their biology and diseases. We would be grateful for any information that would help us to locate the Atlantic projects, to arrange for disease screening if necessary, and to help control any disease problems which may arise.

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Cytoskeletal similarities

SIR—M. Way and A. Weeds in their News and Views article (*Nature* **344**, 292; 1990) suggest that yeast has a simpler cytoskeletal system, lacking redundancy at the gene level, compared with that in the slime mould *Dictyostelium*. But there are at least two lines of evidence indicating that the yeast cytoskeletal network behaves as a parallel distributed processor, as has been considered by D. Bray and J. Vasiliev in their News and Views article on cytoskeleton functioning in the locomotion of *Dictyostelium* amoebae (*Nature* **338**, 203; 1989).

First, the SPA2 protein of the yeast *Saccharomyces cerevisiae* (M. Snyder, *J. Cell Biol.* **108**, 1419; 1989) is associated with actively growing regions of the cell surface. Mutants display either a slightly altered budding pattern (increased numbers of non-axially budding cells) or an inability to stop growing under nutrient-limiting conditions which often results in multiple budding.

Second, a strain containing the null allele for the actin-binding protein SAC1 (P. Novick *et al.* *Genetics* **121**, 659; 1989),

when grown only below about 14 °C, shows defects in the actin-filament distribution.

Hence, these proteins participate in morphogenetic events of the yeast cell, but they are not essential for survival. In terms of parallel processing, these proteins, like those of *Dictyostelium*, could be organized in a hierarchical network.

Partial damage to the parallel processor does not result in a complete loss of function, but in an increased ratio of incorrect decisions. The data suggest an increased number of mutant cell errors. This is obvious, particularly under suboptimal growth conditions which may contribute to increased information noise within the net. Thus, the cytoskeletal systems may operate in a similar mode within both cell types.

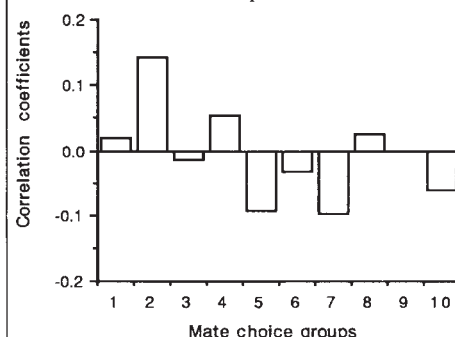
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Pheasant spurs out of fashion

SIR—The report of female pheasants preferring males with longer spurs¹ and the comments thereon^{2,4} prompt me to communicate briefly my findings in British pheasants, obtained from experiments performed in May and June 1988. These birds showed no female preference for longer spurs at all.

The mate-choice pen consisted of a



Male spur length does not correlate with female choice. The figure shows correlation coefficients for a simple regression of female preference within each group.

square enclosure 15 metres by 15 metres, with a 3-metre-long oval-shaped pen built into each side to hold the males. I used 40 non-related males and 40 females, and performed 10 separate trials, each consisting of four males and two pairs of females. All the birds were 1 year old, and the males had been kept in separate pens for 3 months before the trials. The females had not had previous experience of adult male pheasants. I measured female preference by the number of females who solicited copulations from each male in a trial. I also measured this parameter by the proportion of time each female spent within 1 metre of each male.

There was considerable variation in male spur length (not including tarsus width) with the mean at 9.2 millimetres (range 0–12.5 millimetres, standard deviation 2.145). But association of female copulation solicitation with spur length is not significant ($P = 0.8183$; $N = 40$; correlation coefficient = 0.003; standard error = 0.013; see figure). Female preference for spur length, as measured by female proximity within 1 metre of a male was also not significant, but the pattern was different from that of choice measured by copulation solicitations.

Von Schantz *et al.*² measured female preference as females less than 100 metres from a male. I suggest that females could benefit from spending time near a long-spurred male when feeding to obtain the most protection from non-territorial males and predators. Females are known to be considerably less vigilant when in the presence of a male³, and perhaps can afford to be more so if they know the male

is formidable.

Of course, I appreciate that, in practice, proximity may often be the only measure available, and it would indeed be expected to correlate with choice. It is surprising that, in my data, spurs are so insignificant. It is possible that the males from which females solicit copulations are not always the same as those they feed near. The combination of my studies and those of Von Schantz *et al.*¹ suggest either that Swedish pheasants are differently choice-oriented from their British

counterparts, or that measuring female preference by distance from the male rather than by actual copulation solicitation is misleading.

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Retroviral antibodies in Indians

SIR—In February last year, two of us (G. H. and R. W.) took part in the Tapirapico expedition into the rainforest of the upper Mavaca River, a tributary of the Orinoco. We visited three remote Yanomami villages (shabonos), Mishimishiporepithiwe, Washewe and

much lower than the 13.7 per cent reported earlier for Venezuelan Amazonian Yanomami Indians⁹.

These differences reflect either the complete isolation of the population which we have examined or, once again, technical difficulties in analysing sera

ANTIBODIES AGAINST HTLV-1 AND TYPE-D RETROVIRUSES IN YANOMAMIS

Village	Sample No.	Virus	Antibody-positive individuals	
			No. positives	Individual (age, sex)
Mishimishiporepithiwe	112	HTLV-1	1	10 M
		type-D	2	8 M, 8 F
Washewe	82	HTLV-1	2	35 M, 7 F
		type-D	0	
Peripapuey	69	HTLV-1	0	
		type-D	3	30 M, 35 F, 25 M

Peripapuey, took 263 blood samples, representing about 90 per cent of the inhabitants of these villages, excluding very small children. We prepared sera in the base camp and then shipped samples to Germany.

There, we examined the samples for antibodies against five retroviruses: human immunodeficiency virus (HIV) types 1 and 2; human T-lymphotropic virus type I (HTLV-1)^{1,2}; human foamy retrovirus (HFRV)³, and simian type-D retrovirus (SRV-1)⁴. We had earlier demonstrated the specificity of our HIV- and HTLV antibody tests using African sera^{1,2}. We found antibodies against HTLV-1 in only three samples and against SRV-1 in only five samples. We observed no obvious signs of disease that might be related to HTLV-1 or type-D virus infection. In addition, sera from 18 military personnel and 17 Makiritare indian workers from the base camp were negative in all five assays.

Our results confirm and extend an earlier report⁵ but differ from observations by Volsky and co-workers, who described HIV serum antibodies in Venezuelan Pemón and Yanomami Indians^{6,7} and also the isolation of an HIV-related virus from such a sample⁸. Moreover, the prevalence of HTLV-I antibodies in our sample was

from tropical regions^{1,3}. In addition, a type-D retrovirus has been isolated from squirrel monkeys (*Saimiri sciurus*)¹⁰. However, the significance of serum antibodies in Yanomamis to type-D viruses remains to be determined.

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Gyro results

SIR—I read with great interest the analysis by S. H. Salter (*Nature* **343**, 509–510; 1990) of the gyroscope weight loss reported by H. Hayasaka and S. Takeuchi (*Phys. Rev. Lett.* **63**, 2701–2704; 1989). Salter says that the straight lines of weight loss versus rotational frequency do not meet at a point corresponding to zero weight change for zero rotation. But they do; Salter evidently overlooked the fact that the scale on the abscissa does not start from zero, further regression analysis (done by me) confirms that both lines meet at the origin.

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SIR—Hayasaka and Takeuchi recently reported¹ anomalies in the weight of rotating gyroscopes. Their results were peculiar in at least two respects. (1) A gyroscope whose angular momentum ω pointed down reportedly exhibited a weight reduction proportional to $|\omega|$, whereas one with ω directed upwards exhibited no weight anomaly. (2) The effect was large, the change in gravitational mass of a gyro spinning 'downwards' being

$$\frac{\Delta m(\kappa)}{m} = \kappa \frac{r_{eq} \omega}{g} \quad (1)$$

where r_{eq} is a characteristic radius of the gyroscope, and the coefficient $\kappa = -2 \times 10^{-5} \text{ s}^{-1}$. These effects have not been confirmed in two subsequent experiments using macroscopic gyroscopes^{2,3}.

Much tighter limits on such anomalous behaviour can be obtained from microscopic gyroscopes. If the effect reported by Hayasaka and Takeuchi applies at the microscopic level, it will not average to zero for unpolarized test bodies. An unpolarized test body will exhibit gravitational mass loss because the weight decrease of particles whose orbital angular momentum l is down is not compensated by a weight gain of particles whose l is up.

A recent comparison of the acceleration of beryllium and copper test bodies in the Earth's gravitational field⁴ showed no difference between gravitational and inertial masses at the level $\Delta m/m \leq 1.0 \times 10^{-11}$. Because the mean $|l|$ of the electrons and nucleons in copper is higher than it is in beryllium, the null results of the beryllium/copper comparison indicate that the inertial masses of particles with l up and l down must differ by precisely the same amount as their gravitational masses. The energies of atomic and nuclear levels are proportional to the inertial masses of the electron and nucleon, respectively. Hence if Hayasaka's and Takeuchi's effect occurs on a microscopic scale there will be anomalous

splitting of energy levels. But high-precision atomic and nuclear spectroscopic measurements set stringent upper limits on such effects.

Consider, for example, the case of the $2S_{1/2} \rightarrow 3P_{1/2}$ transition in the hydrogen atom. For simplicity, it will be discussed using semi-classical theory in which $r_{eq}\omega = l(m\langle r \rangle)$ where m is the inertial mass of the electron and $\langle r \rangle = n^2 a_0$ is the Bohr radius (n is the principal quantum number and $a_0 = 0.53 \times 10^{-8}$ cm). If equation (1) applies at the microscopic level, the electron gyroscope has

$$\left| \frac{\Delta m_R}{m} \right| = \kappa \frac{l}{mn^2 a_0 g} \sim 4.5 \frac{\hbar}{n^2} \quad (2)$$

and one would expect the $2S_{1/2} \rightarrow 3P_{1/2}$ transition to be split by an energy $\Delta E = 1.5$ eV $\Delta m_R/m \approx 0.76$ eV due to the anomalous inertial mass of one of the magnetic substates of the $3P$ level. This transition has been studied² with a resolution better than the natural linewidth of $\approx 2 \times 10^{-6}$ eV, and no anomalous splittings were observed. Very widely split components of the $3 \rightarrow 2$ transition can be excluded by the fact that the transition rates agree with conventional theory and are inconsistent with the notion that one component of the line is highly anomalous. Thus the upper limit on κ from the electron gyroscope is roughly 4×10^5 times smaller than the value claimed by Hayasaka and Takeuchi.

A considerably more stringent limit comes from nuclear gyroscopes. The Mössbauer effect, for example in ^{57}Fe , cleanly resolves all the hyperfine states corresponding to the allowed orientations of the nuclear angular momentum (see ref. 6, for example). If equation (1) applies, one can estimate $\Delta m_R/m \approx 8.6$, where $l = \hbar$ and $\langle r \rangle \approx 5$ fm for the $2p$ neutron that 'jumps' in the Mössbauer transition. The energy splitting of the 14-keV γ -ray due to the splitting of the neutron inertial mass would be roughly

$$\Delta E \approx 30 \text{ MeV} \frac{\Delta m_R}{m} \approx 2.6 \times 10^8 \text{ eV} \quad (3)$$

It is known that there are no anomalous splittings in the ^{57}Fe transition greater than the natural linewidth of $\Delta E \approx 4.6 \times 10^{-9}$ eV. This is nearly 17 orders of magnitude below the value one expects if Hayasaka's and Takeuchi's effect applied in the microscopic realm.

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Radiation hazards and leukaemia

SIR—The articles by M. J. Gardner *et al.* (*Bri. med. J.* **300**, 423–429 and 429–434; 1990), on the possible linkage between high exposure to radiation of fathers working at Sellafield and leukaemia clusters in their offspring, highlight the need for more careful monitoring of links between both occupational radiation and high natural radiation exposure, and disease in families of certain groups.

Gardner *et al.* suggest that the lack of a relationship between massive doses of radiation in survivors of the Hiroshima atomic-bomb explosion and leukaemia in their children may be because the genetic linkage requires a cumulative effect of smaller radiation doses over a long period. If such a linkage is possible, then it is surely imperative to study families where one or both parents may have been exposed to either of these types of radiation before conception, and where a child develops leukaemia. In this respect, data are required on families of various groups: reprocessing-plant and reactor workers, tin miners in southwest England, airline pilots with high exposure to cosmic rays, and those exposed to high natural radiation from radon, for example.

In the case of natural radiation exposure, for example, R. H. Clarke and T. R. E. Southwood (*Nature* **338**, 197; 1989) pointed out that in parts of Cornwall in southwest England individual exposure levels to the α -emitter radon and its daughters can rise to doses greater than 20 millisieverts (mSv) a year, a high proportion of which comes from indoor radiation. The average individual exposure to radon in Cornwall in 1988 was given as 7.8 mSv a year compared with the national average of 2.5 mSv. This compares with average occupational exposure rates in the same period for workers in reactor plants of less than 1 mSv a year and of about 5 mSv for workers in reprocessing plants. Although no data were given for exposure to cosmic rays of airline pilots, Clarke and Southwood suggested that airline travellers received slightly greater annual exposure to cosmic rays than the average for individuals in the United Kingdom as a whole. The figure of greater than 20 mSv a year for some people in southwest England can also be compared with the data of Gardner *et al.* for the four Sellafield fathers with children who developed leukaemia; the four had average exposures over several years of 14.6, 27, 27 and 7.5 mSv, respectively.

Given the kind of data available on indoor exposure to natural radiation in parts of Cornwall and elsewhere, what data do we have on leukaemia among children where the mothers have been exposed before pregnancy to high levels of radiation over comparable periods of time? If such data are available, how do

they compare with data for workers in other high-exposure industries? Information of this kind is clearly important if genetic linkages between disease, and exposure of a parent to high natural or occupational radiation, are to be substantiated.

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Protein instruction

SIR—Townsend *et al.*¹ recently reported the unexpected influence of peptides on the expression of complexes of class I MHC heavy chain with β_2 -microglobulin (β_2m) and, in that context, Parham² defended a surprising instructional theory of protein folding. Attractive as they may appear, we should remember that such theories have vanished from immunology because they do not make much sense at the molecular level.

In water, the folding of large proteins is driven by large entropic terms arising from the need that their hydrophobic cores should be hidden³. Thus, folding is a spontaneous process, faster than chain synthesis. In contrast, protein-protein interactions are mediated by short-range low energy forces (electrostatic, hydrogen bonds, dispersion). Thus, stable complexes require the cooperation of many individual interatomic interactions.

It follows that two protein molecules will assemble together only if their overall shapes and force fields are already quite complementary before collision. Indeed, conformational changes might further stabilize the initial complex, but a better than random affinity must pre-exist the actual collision of the molecules: there is induced fit, not instructed folding.

The case of the peptide/class I MHC interaction raises even more difficulties. First an isolated peptide fluctuates among a large number of flexible conformers, so that its 'instructive' value must be poor. Second, the numerous class I amino-acids in a position to bind the peptide in the stable complex are interspersed throughout the polypeptide chain (at locations 5, 7, 9, 26, 59, 63, 66, 70, 73, 95, 97, 99, 114, 116, 152, 156, 163 — see refs 4, 5) in such a way that no local interaction with the ligand could occur from an appreciably unfolded conformation.

Indeed, the results of Townsend *et al.* can still be interpreted within the standard, selectionist framework. In solution, the class I MHC heavy chains are dynamically distributed among a number of conformations of similar energy and topology, some of them with an appreciable affinity for a given peptide epitope. As a collision

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occurs between two complementary forms of the peptide and the MHC heavy chain, a stable complex is formed. An allosteric transition⁶ could then be invoked to explain a preferential interaction between these peptide-loaded heavy chains and β_2m . In turn, the binding of the β_2m moiety will further stabilize the peptide/class I complex (of which the spontaneous dissociation is very slow⁷), so allowing the translocation of a peptide-loaded class I/ β_2m complex to another cellular compartment. Because of the mass action law, the presence of suitable ligand peptides will then progressively drain out the uncomplexed form of the class I heavy chain.

Other peculiar features can also be explained in standard ways. Thus the apparent irreversibility of the β_2m /class I heavy-chain dissociation⁸ may be due to the fact that the association rate, obeying a trimolecular kinetics, may be undetectable in the diluted *in vitro* conditions. Moreover, biosynthetic studies showing that class I heavy chain must associate with β_2m within 30 min of translation⁹ may result from a competition with the glycosylation pathway. Thus, even though the mechanism of antigen presentation exhibits unexpected features, there is as yet no need to dig out instructional theories from the fossil records of immunological thinking.

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PARHAM REPLIES — In trying to understand antibody specificity, immunologists historically devised various instructional theories. A common theme was the involvement of foreign antigen in forming the three-dimensional structure of antibodies. It is this aspect of instructional models which, I suggested in my News and Views article¹ "A profitable lesson in heresy", had relevance to antigen presentation by MHC molecules, an idea also proposed by J. C. Howard in summing up last year's Cold Spring Harbor symposium. This view is based on a synthesis of many data indicating that under physiological conditions class I MHC molecules do not assemble and get out to the cell surface to stimulate T-cell responses unless they are associated with bound peptides and in appropriate conformations. Thus MHC molecules, unlike antibodies and T-cell receptors, are dependent for their structure and biological function on association with putative antigens.

In Paris, it seems that some are still eager to burn heretics. Claverie accuses me of "defending a surprising instructional theory of protein folding" that he believes contradicts the well-established rules of protein folding. In part this is a semantic issue. In saying that "MHC

molecules fold up around the peptides they present to T-cell receptors", I meant that peptides contribute to the acquisition of tertiary and quaternary structure and not to formation of basic elements of secondary structure, as apparently understood by Claverie.

More distressing problems exist with Claverie's exposition of what proteins can and cannot do, which appear to stem from reliance on a chemistry text published in 1979 and ignorance of newer results suggesting that protein folding *in vivo* is not necessarily identical to that observed *in vitro*. The dichotomy between protein folding as viewed by the physical chemist and as viewed by the cell biologist has been recently reviewed² and it is clear from a spectrum of biological systems that the biosynthesis, membrane translocation and subunit assembly of certain proteins involves molecular chaperones³ that control their folding and unfolding. Thus, folding of class I MHC heavy chains in the complex environment of the cell need not be spontaneous and faster than chain synthesis, as concluded by Claverie.

The historical debate about antibody specificity was between instruction and selection; the first of these alternatives postulated one primary structure folded under the influence of antigen to give multiple conformations with different combining-site specificities; and the second correctly argued for numerous primary structures from which an antigen could select those with sufficient combining-site affinity. In denouncing instruction for MHC, Claverie turns to its historical foe and claims that "the results of Townsend *et al.* can still be interpreted within the standard selectionist framework". His suggestion that both peptides and class I MHC heavy chains have multiple interconverting conformations, that complexes will form between particular conformations, and that their stabilization may have mass-action effects is reasonable. But surely it is more a kinetic description of instruction — different peptides binding conformations of the same MHC sequence — rather than of selection, with its implication for distinguishing distinctive primary sequences. Claverie appears to have confused selection in the general sense with selection in its specific, immunological sense.

In his last paragraph, Claverie criticizes the suggestion that conformational requirements for bound peptide can explain previously unexplained properties of class I MHC molecules, and implicitly challenges the now widely held belief that bound peptides are essential components of normal, functional class I MHC molecules. Claverie brings no news to this subject and his alternative explanations do nothing to perturb my views. That rate-limiting glycosylation can explain why class I MHC heavy chains must associate

with β_2m -microglobulin (β_2m) within 30 min of translation is amenable to experiment, and one should perhaps encourage Claverie to test his hypothesis.

In the case of denaturation/renaturation, Claverie's grasp of the data appears tenuous. Our own experiments showed that unfractionated but denatured class I molecules can be efficiently renatured whereas reconstituted class I heavy chains and β_2m cannot; the difference cannot be explained in terms of protein concentrations as they were kept identical. One can also take issue with the suggestion that the association of heavy chain and peptide involves trimolecular kinetics, which I assume means a three-body collision. This is unlikely to be a significant pathway compared to the initial formation of a bimolecular complex followed by binding of the third component — a mechanism in fact mooted by Claverie in his previous paragraph.

Claverie and colleagues have keenly followed Alain Townsend's experiments and have previously developed a "peptidic self" model of the immune system as a "logical generalisation based on an interpretation of results by Townsend *et al.*"¹¹. This model emphasized a role for MHC molecules in determining the conformation of antigenic peptides as presented to T cells which perhaps explains Claverie's extraordinary reaction to consideration of the reciprocal effect.

On reading "that such [instructional] theories have vanished from immunology because they do not make much sense at the molecular level", it is worth considering that much of the vigour of immunology has stemmed from the discovery of phenomena such as MHC restriction that did not make much sense within the prevailing molecular framework. Application of molecular biology and new technologies to the study of protein folding and stability is now producing results that significantly challenge both the existing dogma and generalizations based on analysis of small numbers of simplified and model systems¹². MHC-peptide interactions have unusual features for study and could provide unique contributions to this area.

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The man and the myth

Adam Kuper

The Kon-Tiki Man — Thor Heyerdahl. By Christopher Ralling. BBC: 1990. Pp.335. £16.95.

SWEDES like to joke that the thinnest book in the world is the *Norwegian Who's Who*, but in Thor Heyerdahl the book includes perhaps the most famous anthropologist of the century. Heyerdahl's book on the Kon-Tiki expedition, which sold more than 20 million copies, has probably outsold all other anthropological books combined. This is naturally somewhat galling to other anthropologists (perhaps most especially to Swedish anthropologists).

The present book, by a TV director, is intended to accompany a television series on Heyerdahl's life and voyages. Ralling represents Heyerdahl not only as a great scientist, the hammer of the orthodox, but also as a mystic, prophet of the Greens and as a pioneering internationalist (who nailed the flag of the United Nations to the mast of some of his more unusual craft) and burnt one boat, as he informed the UN Secretary-General in a telegram, in protest against the horrors of the world. In fact, there is something very Norwegian about Heyerdahl, and that may be part of his appeal.

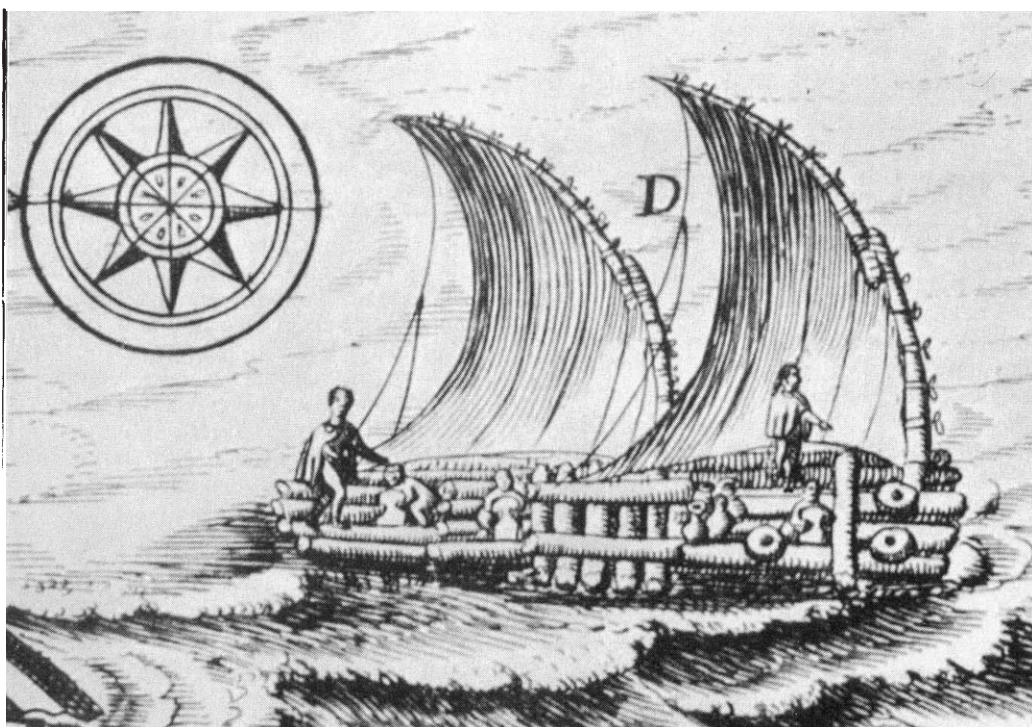
But first, the theories. At the turn of the century there were two main organizing ideas in anthropology. One, a more-or-less darwinian evolutionism, understood the various cultures of the world as representing stages in a single trajectory. Darwin himself recorded, "on first seeing a party of Fuegians on a wild and broken shore" that "the reflection at once rushed into my mind — such were our ancestors. . . They possessed hardly any arts, and like wild animals lived on what they could catch; they had no government, and were merciless to every one not of their own small tribe." If the Fuegians represented our early ancestors, on this view, then the Inca might correspond to the Greeks in level of civilization, the Ming Chinese to mediaeval Europe, and so forth. Every human community was engaged on a climb onwards and upwards along the same path. What drove them on might be intellectual development, economic progress, moral improvement or whatever other motor of cultural evolution appealed to a particular theorist. It need hardly be said that there is nothing

very darwinian about this so-called evolutionism.

The other theory denied that change and development were endogenous. They resulted from contact, trade and, especially, migration and conquest. This thesis was developed especially in Germany and by geographers. Its proponents mapped the distribution of traits — fire-lighting devices, decorative styles, fish-nets — and

some time, and a Swedish scholar, Baron Erland Nordenskjöld, a specialist on ancient America, opined that the direction of influence may on the contrary have been from the Americas to the Pacific Islands.

There were, however, a great many objections to all of these models even before the modern development of archaeological and linguistic research. One very down-to-earth objection concerned the means of transport. How had hordes of immigrants travelled along those bold arrows across the seas? Heyerdahl took up Nordenskjöld's hypothesis, and prepared to demonstrate that a very crude — notionally 'precolumbian' — raft could indeed be sailed from South America to Polynesia. In 1947, he and five



An early seventeenth century drawing of a balsa wood raft off the coast of Peru.

deduced the population movements which had distributed them. This is the school of thought which has landed us with history books covered in thick arrows tracing speculative population movements of ancient 'races'.

Diffusionism has always had a fatal attraction for amateurs, and shortly before World War I (when what Julian Huxley called "the eclipse of Darwinism" spread to England) the anatomist, Elliot Smith, began to advocate an extreme version of the thesis. All civilization can be traced back to ancient Egypt, and from there the spread of civilization around the world can be traced by the appearance of a complex of diagnostic traits, notably sun-worship, megaliths and mummification. These travelled from the Middle East to India, on to Indonesia, then to the Pacific, and finally to the Americas. Competitive versions of this scheme were peddled for

companions carried out this extraordinary feat, sailing across about 8,000 kilometres of ocean in three and a half months on a balsa-wood raft.

This expedition provided the model for others. Heyerdahl went to Easter Island to see the great stone sculptures and showed that quite primitive craft could pass between Easter Island and the South American mainland. The next move, logically enough, was to prove that a reed raft — perhaps rather like those used in the ancient Near East — could be sailed across the Atlantic, from North Africa to the Caribbean. This was the rationale for the voyages of *Ra* and *Ra II*. Finally, Heyerdahl sailed another reed craft from the mouth of the Tigris, through the Gulf to Karachi, and then westwards to Africa, landing in Djibouti at the mouth of the Red Sea.

These expeditions certainly removed

From The Kon-Tiki Man

one objection to the early diffusionist theorists — it was, apparently, possible to sail one of these frail craft across the oceans. But by this time, other forms of evidence were readily available which demonstrated that Oceania, for instance, had without question been colonized from Indonesia, and that the remote Pacific islands like Easter Island had been colonized from central Polynesia. Food crops and perhaps other culture items may have been borrowed from the Americas, but there was never any significant population movement from America to the Pacific until modern times. Nor was there a single source of high culture in the world. Moreover, cultural change was no longer solely or even primarily attributed to migrations. In many parts of the world it was clear that changes followed a local course, and that (contrary to the speculations of the evolutionists) these could take divergent routes, as, indeed, a

true darwinian would have anticipated.

But Heyerdahl's missives are not addressed to the anthropologists, and his fundamental message is not really about ancient Egypt or Peru. The message is a myth. All human culture is one in origin, all men are brothers, nature is pure but it must be mastered, and it can be, with courage and using the tools of ancient civilization. This is a message which can be traced, by any good, amateur diffusionist, to the cultivated, puritanical, provincial world of pre-war Norway, from where it has been carried across Europe, and indeed across the world, by a race of fair-haired ecologists, development experts, missionaries and, of course, Thor Heyerdahl himself. The BBC should have broadcast this series in its religious slot. □

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From East and West

John E. Midwinter

Optical Computing. Edited by B. S. Wherrett and F. A. P. Tooley. *SUSSP Publications, Edinburgh University Physics Department: 1989. Pp. 314. Distributed by the Institute of Physics, £45.*

Optical Processing and Computing. Edited by H. H. Arsenault, T. Szoplik and B. Macukow. *Academic: 1989. Pp. 493. £42.50, \$59.50.*

HERE are two hard-cover volumes on the general topic of optical computing. The first, by Wherrett and Tooley, stems from a NATO Advanced Study Institute held in 1988. The second, by Arsenault *et al.*, is a conventional edited volume in which each chapter is written by a different author. However, and perhaps of particular interest to Western readers, about half of these are from Soviet authors.

Both books start with chapters by Goodman of Stanford University. In *Optical Computing*, Goodman's historical essay provides an admirable basis against which to view both books. Goodman traces the thinking of optical computing from the early theories on the resolution, imaging properties and coherence of optical systems of Abbe and the recognition that the near- to far-field power distributions are intimately related to the Fourier transform and to Zernike's work in the study of phase objects, such as in the phase-contrast microscope.

This was followed during the late 1950s and early 1960s by work on coherent analogue processors which explicitly

exploited the Fourier-transform relation to implement the functions of frequency filtering, correlation, convolution and so on. In most of these early processors, the 'data' had to be fed into the optical system using a photographic film, representing a major engineering problem for real-time processing systems. Because the main application of the technique was radar processing, this problem was very serious. From those early days, one can trace the attempts to use acousto-optic modulators and spatial light modulators as data-input devices to overcome these problems, yet despite advances in optical technology, it seems that 'silicon' has always managed to stay ahead.

One obvious problem with all analogue signal processing is the need to handle large dynamic range signals, something

New Journals

This year *Nature's* annual new journals review supplement will appear in the issue of 11 October. Publishers and learned societies are invited to submit journals for review, taking note of the following criteria:

- Journals which first appeared after June 1988, and which issued at least four separate numbers by the end of April 1990, will be considered for review. The deadline for submission is the end of June.
- Journals covering any aspect of science are eligible, although those dealing with clinical medicine, engineering and pure mathematics are excluded, as are publications of abstracts.
- Frequency of publication must be at least three times a year.
- The main language used must be English. Translation journals in English are eligible.

When submitting journals for review, please send at least four different issues (the first, the most recent and any two others) of each title as soon as possible to: Book Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, UK or 1137 National Press Building, Washington DC 20045, USA.

that is particularly difficult in optical systems. Hence attention has increasingly shifted to digital-processing schemes using optics as a possible solution. Of course, this brings the optical processor closer still to its electronic digital equivalent and again invites a comparison which is usually not very favourable. Undoubtedly the biggest problem facing all optical computing proposals is the astonishing success of electronic approaches. Hence it is perhaps disappointing to see that the contributors in neither book make a serious attempt to relate what optics can or might do to what electronics already does — although of the two, *Optical Computing* is superior in that it includes one chapter dealing with the technology trends for VLSI circuits and another discussing current developments in electronic parallel processing.

For a reasonably well-balanced overview of the current topics in optical computing, my choice is the volume edited by Wherrett and Tooley. The selection of speakers at the original meeting was good, giving balanced representation to the many strands of activity that fall within this broad field. There are good reviews on both the analogue and digital aspects, on many of the key devices currently being studied and on the system and architectural issues that are attracting debate.

The book edited by Arsenault *et al.* seems less well balanced. A very practical introductory chapter on optical interconnects is followed by a series of contributions (15 in all) in which the emphasis is fairly heavily on analogue processing, a field which has been 'around' for at least 30 years but which seems to have delivered rather little. Accordingly, I was left with the strong impression that there is little that can be described as "up to the minute" in the book although it does share with *Optical Computing* the obligatory (!) chapter on neural networks from the outstanding group at Caltech led by Psaltis. Of the Soviet work featured, only the chapter describing studies of biopolymers (bacteriorhodopsin) was new to me, although I would not claim to be fully *au fait* with every detail of Soviet research.

In summary, then, it seems clear that for a balanced assessment of optical computing today, one would be well advised to read Wherrett and Tooley's book. The book by Arsenault *et al.* supplements this in some respects, for example in a detailed review of photo-refractive materials by Tanguay, but is probably going to be of prime interest for the view it presents of work in the Soviet Union that has not been well reported at conferences. What neither will do with ease is explain to the reader how or when optical technology is likely to impinge seriously on the established might of electronics. □

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Community work

Michael J. Crawley

Ecological experiments: Purpose, design and execution. By Nelson G. Hairston Sr. Cambridge University Press: 1990. Pp. 370. Hbk £30, \$53.50; pbk £15, \$24.95.

IN HIS presidential address to the British Ecological Society in 1957, George Varley suggested that there were alternating modes of population regulation in successive trophic levels. If one trophic level, say plants, was limited by competition then the next trophic level (herbivores in this case) must be limited by natural enemies. At about the same time, but unaware of Varley's work, Nelson Hairston prepared a paper with Frederic Smith and Larry Slobodkin on the relative importance of competition and predation in different trophic levels. Their paper was rejected by *Ecology* and eventually appeared in *American Naturalist* in 1960, since when it has become one of the citation classics of community ecology.

Hairston *et al.* reasoned that plants, natural enemies and decomposers are all limited by competition for food, but that herbivores are kept scarce by natural enemies (they did not compete for food and have little impact on the abundance of plants). In brief, "the world is green and we're not making coal". The hypothesis was darwinian both in the breadth of its vision and in the fact that it derived from natural history observations rather than from controlled experiments.

Despite repeated attacks during the intervening 30 years (the world is obviously not always green, herbivores sometimes do have important effects on plants, and predators do not always compete for resources), the hypothesis has stood the test of time remarkably well. As Hairston shows in his new book, there is no compelling evidence against any of the argument's central tenets.

Hairston's main theme here is that manipulative field experiments offer the best (perhaps the only) means of understanding ecological communities. He makes the important point that robust generalizations about the relative importance of competition and natural enemies are only likely to emerge if the outcome of experiments is considered one habitat at a time sessile animals compete directly with plants (for example, in rocky intertidal habitats where space is often the limiting resource whereas there is no equivalent process in muddy intertidal or in terrestrial habitats). To this end, he draws examples from forests, successional communities, deserts, fresh water and marine communities, and each chapter describes a selection of experiments that have been carried out on how populations

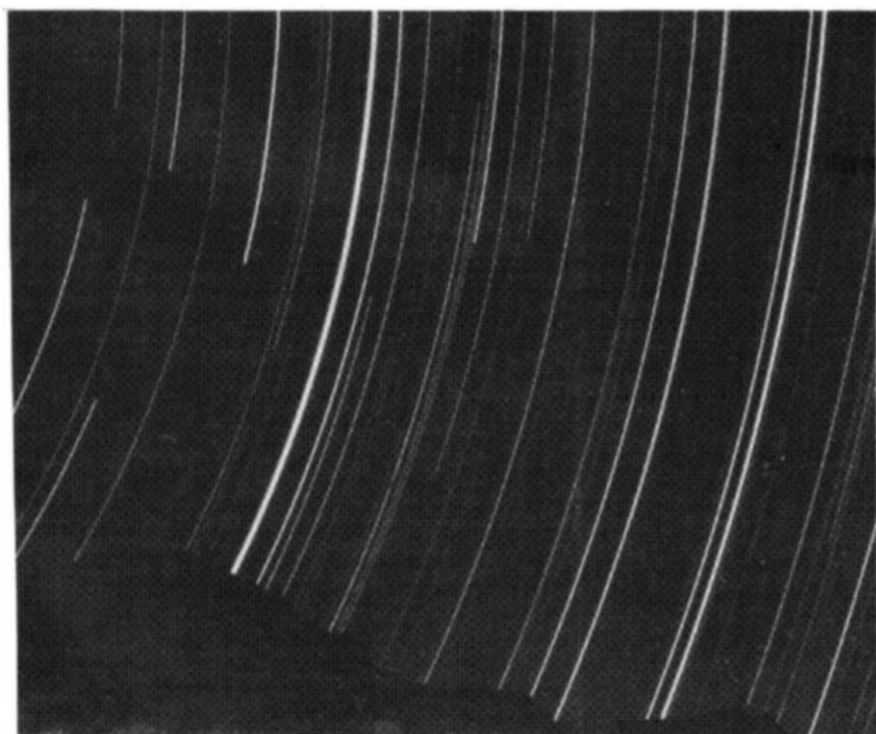
of decomposers, plants, herbivores and predators are regulated.

Nature has no stake in being understood by ecologists, and even the simplest questions tend to have complicated answers. This has meant that good field tests of competing hypotheses about population regulation are few and far between. The result is that our detailed knowledge is hideously fragmentary (it is like having one or two pieces from lots of different jigsaws) and generalizations are inevitably based on case histories that derive from a wholly unrepresentative set of species (those that are reasonably easy to work with) and a somewhat esoteric collection of habitats (places where ecologists like to do their field work). In no case has population regulation been studied in simultaneous field experiments on all the components of the same ecosystem (plants, herbivores, carnivores and decomposers). The task of carrying out simultaneous, long-term, well-replicated, experimental studies on all the important components of any real ecosystem would be the ecological equivalent of sequencing the human genome.

One of Hairston's aims is to inspire better ecological experiments. He does this by describing examples of both good and bad designs, and he pulls few punches in exposing the shortcomings of the ill-designed studies (one extreme case, published in the *Journal of Animal Ecology* in 1972, had no control, no replication, no randomization and no statement of the initial conditions). Although it is true that ecologists have never been renowned for the quality of their experimental designs (for example, the high frequency of 'pseudoreplication' exposed by S. H. Hurlbert in 1984), it should be said that contemporary experimental ecologists, brought up in the post-Hurlbert era, are much better informed on matters of experimental design (most of Hairston's examples of poor design come from studies published before 1985).

Like most advocates, Hairston could be accused of over-selling his product. For example, he has little time for observational studies (comparative surveys or 'natural experiments') and the contributions of ecological theory get short shrift. Thus, while you would be unlikely to choose him as your guide to the strengths of the comparative method or as an advocate for theoretical ecology, when it comes to writing about ecological experimentation Hairston is unbeatable. This is the best ecology book to appear in several years. It is instructional, entertaining and unmatched in the breadth and distinction of its scholarship. □

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Time-exposed star trails over the barren Cabeza Prieta Mountains (1985), reproduced from *Desert Heart* by W. K. Hartmann. The volume charts both the natural history and cultural history of the Sonoran Desert, southwest United States. Published by Fisher, price \$35.

The devil and the deep blue sea

Paul Mellars

Science-based Dating in Archaeology. By M. J. Aitken. Longman. 1990. Pp. 274. Hbk £22; pbk £12. Distributed in the United States by Addison Wesley \$53.75.

FEW developments have had a greater impact on archaeology over the past forty years than the advent and refinement of scientific dating techniques. The initial application of the radiocarbon method, for example, effectively doubled the documented time range of prehistoric farming communities, while the development of potassium-argon dating had much the same effect on studies of early human origins. The impact of the 'second radiocarbon revolution' (arising from systematic tree-ring calibration of 'conventional' radiocarbon dates) was almost equally dramatic, and called for a radical reappraisal of most of the conventional reconstructions of prehistoric trading networks and cultural 'diffusion' patterns across Europe.

Confronted by these developments, of course, the archaeologist is often caught between the devil and the deep blue sea. New dating techniques emerge, new batteries of dates are thrown into the archaeological arena and, not infrequently, the scientific specialists themselves often seem to be caught up in debates over the relative reliability or significance of the results produced by different dating methods. The archaeologist is left with the task of finding his way through the array of dates produced, and trying to make some sense of the results in terms of potentially conflicting models for the development of early human societies.

Martin Aitken's review of *Science-based Dating in Archaeology* provides just the kind of lucid, well-balanced view of these new dating methods which archaeologists have long been waiting for. The author is one of the most respected authorities in this field, and has himself made significant contributions in several different fields of scientific dating — particularly those of archaeomagnetism and the recently developed technique of thermoluminescence. As he points out in the preface, Aitken has written the book primarily with the needs of the professional archaeologist in mind. But the volume will be equally useful to students, and will be no less intriguing to professional physicists, palaeobotanists and geologists as an illustration of how their disciplines have been brought to bear on the problems of unravelling the human past.

The coverage of the book is certainly wide-ranging and comprehensive. After

discussing the contributions of the biological and Earth sciences (as for example in the use of pollen analysis and oxygen-isotope studies of deep-sea cores), Aitken comes firmly to grips with the more basic 'absolute' methods of dating, such as radiocarbon, potassium-argon, thermoluminescence, electron spin resonance and uranium series. The author's aim is to make all these methods comprehensible to a non-specialist audience. How far this aim is totally achievable is, no doubt, a matter of debate, but the book comes closer to this goal than most earlier textbooks in the same field. At the same time,

over anyone's eyes. In the long section on radiocarbon dating, for example, Aitken draws attention to the inherent problems of dating very old samples (more than 20,000 years old) and illustrates how even minuscule quantities of modern contaminants can easily clip several thousand years off the real age of the dates produced. Aitken is equally cautious in discussing some of the more recent techniques, such as thermoluminescence and electron-spin resonance, and again draws attention to the wide range of variables that need to be fully controlled ('environmental' radioactivity, moisture content of deposits,

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

The Oxford radiocarbon accelerator is able to date samples up to 100,000 years old when used in conjunction with a high-energy mass spectrometer.

Aitken has kept the needs of more specialist readers clearly in mind. His footnotes to different chapters provide detailed documentation of some of the more technical and mathematical aspects of the various methods, whereas the bibliographies provide an impressively wide-ranging review of the specialist literature in each field. The illustrations have been equally well chosen to show both the basic scientific principles of the different methods, and the kinds of results which have been achieved in specific archaeological applications.

One of the most impressive and refreshing features of the book is the author's frankness in stressing the limitations — and in some cases potential ambiguities — of many dating methods in current use. Clearly, archaeologists are totally at the mercy of their scientific colleagues for the reliability and accuracy of the dates they use, and they have a right to expect the scientists providing the dates to be their own most severe critics. No one could accuse the present book of pulling the wool

variable rates of uranium uptake and so on) to provide any kind of 'absolute' confidence in the results achieved. Above all, Aitken stresses the need for a wide-ranging integrated approach to dating, where the results from several, independent, complementary techniques can be compared and evaluated by the archaeologist. All this is highly laudable, and provides just the kind of hard-headed, critical approach to geochronology which archaeologists are fully entitled to expect from their scientific colleagues.

But this is in no sense a negative or pessimistic text on the problems of archaeological dating. Clearly, some outstanding progress has been contributed by the natural sciences during the past few decades. It is a tribute to Aitken that he communicates much of the optimism and excitement of this work, without making premature or exaggerated claims that all inherent problems have been resolved. □

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Ice-core record of atmospheric methane over the past 160,000 years

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Methane measurements along the Vostok ice core reveal substantial changes over the past 160,000 years which are associated with climate fluctuations. These results point to changes in sources of methane and also show that methane has probably contributed, like carbon dioxide, to glacial-interglacial temperature changes.

MEASUREMENT of the composition of the air in bubbles trapped in polar ice is the most direct way to reconstruct the past atmosphere and the only one available for methane (CH_4). The ice record previously revealed that the CH_4 concentration was only ~ 700 p.p.b.v. (parts per 10^9 by volume) two to three centuries ago, that is, less than half its present level of $\sim 1,700$ p.p.b.v.¹⁻⁴. More recently, two sets of results showed that atmospheric CH_4 doubled when going from full glacial conditions (350 p.p.b.v.) to interglacial periods (650 p.p.b.v.)^{5,6}.

Our study extends the record of past atmospheric CH_4 over the full climate cycle back to ~ 160 kyr BP. It is based on a set of measurements performed on the 2,083-m-long ice core (3G core) of Vostok (East Antarctica, $78^\circ 28' \text{S}$ $106^\circ 48' \text{E}$). Four more samples have been taken at depths of 177.4, 544.7, 554.2 and 642.1 m along a new long ice core (4G) drilled ~ 75 m from 3G. The 3G core has already given information about the past atmosphere, in particular temperature^{7,8}, carbon dioxide (CO_2) content⁹, aerosol concentration and chemistry¹⁰⁻¹². The CH_4 profile shows strong variations of past atmospheric CH_4 concentrations in the 350–650 p.p.b.v. range, well below the present atmospheric concentration. These variations are well correlated with climate change deduced from the isotopic composition of the Vostok ice core. Spectral analysis of the record indicates periodicities close to those of orbital variations. We interpret these CH_4 changes to be the result of fluctuations in wetland areas induced by climate changes. We suggest that the participation of CH_4 and associated chemical feedbacks to warming during deglaciations represents about 30% of that due to CO_2 .

Vostok CH_4 results

The air occluded in the ice bubbles was extracted by melting and refreezing the samples under vacuum. Concentrations of CH_4 were measured using a gas chromatograph equipped with a flame-ionization detector and calibrated with an air standard (CH_4 concentration of $1,200 \pm 100$ p.p.b.v.). A complete description of the experimental procedure is given elsewhere⁶. Blank values obtained by analysing standard gas with artificial bubble-free ice showed that the analytical system introduces a CH_4 contamination of 37 ± 25 p.p.b.v. in the initial CH_4 content. After correction of the results, our estimated overall accuracy, including uncertainties in contamination and calibration but not the accuracy of the standard gas, is ~ 40 p.p.b.v. (2σ). In the upper part of the Vostok core (the first 800 m), fractures and thermal cracks may slightly increase CH_4 concentration but this contamination would be < 40 p.p.b.v. according to measurements performed on both fractured and unfractured samples taken at the same depth. Comparison with other ice records, as shown below, also indicates that this potential source of error is small.

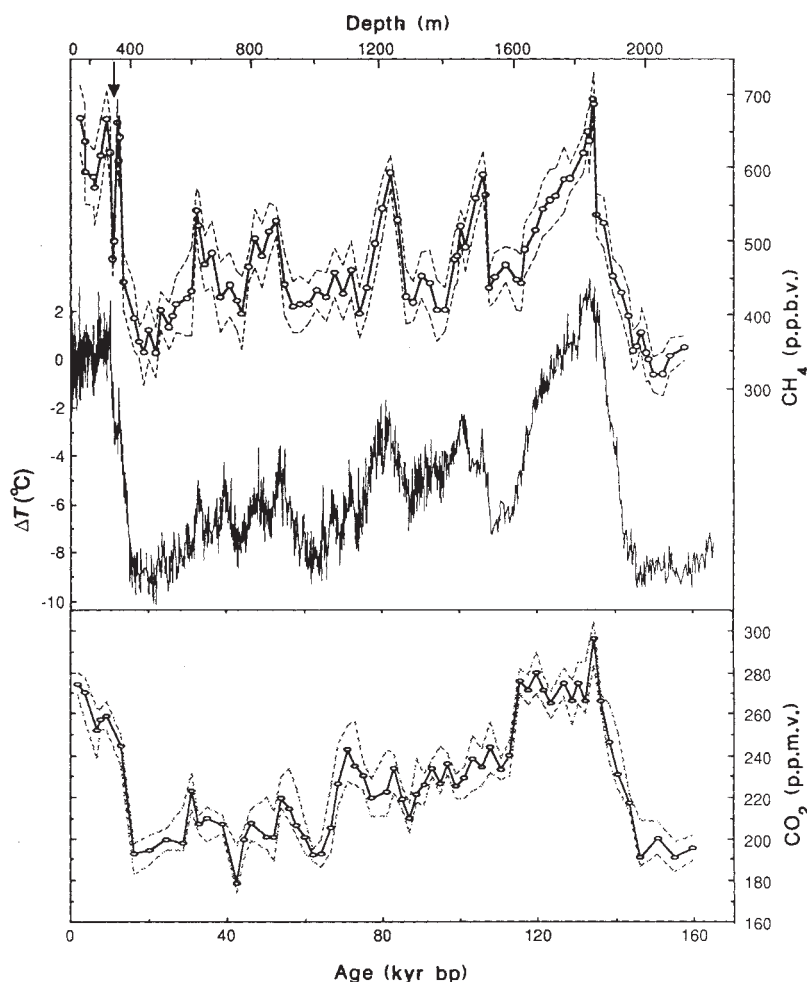
We analysed 156 samples representing 98 depth levels between 149 and 2,064 m, corresponding to a sampling step of ~ 25 m. We include the CH_4 results already published for the Vostok ice core⁶ at seven depth levels. Owing to the air-enclosure process in the ice, the extracted air is younger than the surrounding ice. Also, the composition of the air enclosed in an ice sample represents an average value over several hundred years because all the pores at the depth of enclosure do not pinch off at the same time⁹. Here we calculate the age of the air using the previously described ice chronology⁷ and the age difference between the enclosed air and the surrounding ice⁹. The Vostok ice chronology is under debate, particularly for the period before 110 kyr BP where it departs clearly from the marine chronology^{8,13}. This has to be taken into account when discussing the comparison with other records and furthermore could influence the results of any spectral analysis (see below). Our sampling represents a temporal resolution ranging from 300 yr (close to the average time interval covered by a Vostok air sample⁹) to 3,500 yr. The CH_4 record is plotted against sample depth and estimated age of air in Fig. 1, together with the isotope temperature record derived from the Vostok core⁸.

The CH_4 profile, covering the last climate cycle back to ~ 160 kyr BP (the Holocene, last glaciation, the previous interglacial and the end of the penultimate glaciation), indicates strong variations in the CH_4 content of the past atmosphere. Two main glacial-interglacial transitions appear: one between 2,020 and 1,870 m (150–133 kyr BP) and the other between 450 and 300 m (18–9 kyr BP). In both cases, the CH_4 concentration goes from the lowest values of the Vostok record, ~ 350 p.p.b.v., to the highest values, ~ 650 p.p.b.v. In the depth interval between these two transitions (1,870–450 m), four strong peaks centred on depths of 1,520, 1,240, 880 and 620 m (respectively 106, 82, 52 and 32 kyr BP) stand out above a mean level of 400–450 p.p.b.v. and reach values of ~ 550 –600 p.p.b.v. The latter values are only slightly less than the concentrations observed during the last interglacial period (~ 130 kyr BP) and the Holocene (0–9 kyr BP). The present-day mean CH_4 level of $\sim 1,700$ p.p.b.v. is ~ 2.5 times higher than the maximum value measured in the Vostok ice core.

A striking feature of the CH_4 variations is the large oscillation observed at the end of the last glacial-interglacial transition (marked with an arrow in Fig. 1): after a fast increase from 450 to 650 p.p.b.v. between ~ 380 and 360 m (13–12 kyr BP), CH_4 concentration decreases by ~ 170 p.p.b.v. between ~ 360 and 335 m (12–11 kyr BP), and then reaches again the high value of ~ 650 p.p.b.v. This feature is confirmed by measurements at several depth levels (seven samples for the 'low' value at 11 kyr BP, six samples for the peak at 12 kyr BP).

The transitions between 'low' and 'high' CH_4 values take place over several thousand years. The rates of the fastest changes observed (at the end of the two main transitions and at the onset of the 1,520-m peak) are ~ 0.2 – 0.3 p.p.b.v. yr^{-1} . This rate and the extreme values are low estimates because of the sampling step and of the smoothing of variations linked to the air trapping process in the ice. Nevertheless, these values are comparable to a possible rate of change of ~ 0.3 p.p.b.v. yr^{-1} during the Little Ice Age between AD 1450 and 1750¹⁴. On the other hand, the approximately exponential CH_4 increase (700–1,700 p.p.b.v.)

FIG. 1 Vostok ice-core records. Upper curve: CH_4 record. Circles indicate mean values. The envelope shown has been plotted taking into account the various uncertainty (2σ) sources. The arrow corresponds to the Younger Dryas CH_4 oscillation. Middle curve: isotope surface temperature record as a difference from the modern temperature value (-55.5°C , ref. 8). For comparison, the lowest curve shows the Vostok CO_2 record⁹. The timescale applies to all three records. The depth scale applies only to the CH_4 and CO_2 curves, because of the difference in age between air bubbles and surrounding ice.



over the past 300 years obtained from ice-core analyses and systematic atmospheric measurements during the past 25 years shows a rate of increase from $1.5 \text{ p.p.b.v. yr}^{-1}$ between AD 1700 and 1900 to $17 \text{ p.p.b.v. yr}^{-1}$ today¹⁵. Thus, not only is the present-day CH_4 concentration the largest reached in the past 160 kyr, but it also seems that the present rate of increase was not experienced before anthropogenic perturbation.

Comparison with other ice records. Stauffer *et al.*⁵ have published a record of atmospheric CH_4 including the last deglaciation, obtained from the ice cores of Dye 3 (Greenland) and Byrd (Antarctica). There is good agreement between the general features of these records and those from Vostok, namely, Stauffer *et al.* found a mean CH_4 concentration of 650 p.p.b.v. for the Holocene period (0–9 kyr BP) and values of ~ 350 p.p.b.v. during the Last Glacial Maximum (~ 18 kyr BP). This similarity is corroborated by the Vostok oscillation in CH_4 between 12 and 9 kyr BP which has also been indicated by a single level in the Dye 3 record⁵. Taking into account the differences between the experimental methods used, we have added confidence in the CH_4 measurements from ice cores. It also indicates that any potential error linked to fractures in the upper part of the Vostok core is probably small. An extended comparison covering the period before the Last Glacial Maximum is limited by dating problems and a loose sampling step for the Dye 3 and Byrd records.

A few years ago, the idea that the atmospheric CH_4 concentration remained constant through changes in climate was suggested from the results of Craig and Chou on the Dye 3 ice core¹. This was supported by a single point in the last glacial period showing a CH_4 concentration of 650 p.p.b.v. Our results indicate that in fact this point could correspond to one of the peaks occurring between 20 and 110 kyr BP. Indeed, both the Greenland and Antarctic ice cores indicate that large CH_4 con-

centration changes occurred during climate fluctuations and that glacial and interglacial periods are associated with low and high CH_4 values respectively.

Comparison with the CO_2 record. The Vostok ice core has already provided a profile of atmospheric CO_2 concentration over the entire last climate cycle⁹. The use of the same ice core allows us to compare the CH_4 and CO_2 curves without dating uncertainties.

Although the highest and lowest values in both records are found during the warmest and coldest conditions respectively, during the last glaciation, the two profiles are different. The CH_4 profile exhibits four peaks from a quasi-continuous baseline whereas the CO_2 curve shows a general decreasing trend with a marked break at ~ 70 kyr BP. This difference between the two signals most likely reflects the predominant sources, which are different for CO_2 and CH_4 . The CO_2 variations are suspected to be essentially driven by oceanic changes⁹ whereas the CH_4 cycle, as we discuss below, is mainly controlled by sources from the continental areas.

Spectral analysis. To investigate the potential link between astronomical forcing and methane, we performed a spectral analysis of the CH_4 profile using the multi-taper method (MTM) pioneered by Thomson¹⁶ which provides a high-frequency resolution and a statistical F -test for the validity of amplitude and location of each peak¹⁷. This method is known to be far more reliable than the maximum-entropy method previously applied to Vostok isotope temperature and CO_2 profiles¹⁷. The CH_4 record was linearly interpolated to obtain 500 equally spaced points before carrying out spectral analysis.

The MTM spectrum exhibits four significant peaks at the 90% confidence level (Fig. 2). The periods are 122.0, 41.7, 24.8 and 19.0 kyr. When applying MTM to random curves taken in the 2σ envelope (see Fig. 1), the results indicate that CH_4 spectrum

values are not affected by experimental uncertainties on CH_4 measurements. We also checked the effect on the spectral analysis of changes in ice chronology, using a dust event (event 5 in ref. 13) as a time marker to adjust linearly the Vostok ages older than 110 kyr BP to the marine chronology¹⁸. With this new chronology, the resulting spectrum also shows four significant peaks at 109.9, 38.3, 23.7 and 18.9 kyr with mostly unchanged amplitudes. The four significant CH_4 periodicities seem to be in rather good agreement with orbital frequencies—eccentricity at ~ 100 kyr, the obliquity component at 41 kyr and the two periodicities of the precession of equinoxes at 23 and 19 kyr respectively. Thus the spectral analysis supports a fundamental relation between orbital climate forcing and CH_4 concentration. At low frequencies, however, the 100-kyr component, which generally dominates palaeoclimate spectra¹⁹, was not predominant in the CH_4 spectrum whatever chronology we used. This feature may help to constrain the CH_4 -cycle interpretation as discussed below.

Comparison with temperature profiles. The continuous record of deuterium content ($\delta\text{D} = [(^2\text{H}/^1\text{H})_{\text{sample}}/(^2\text{H}/^1\text{H})_{\text{standard}}] - 1$) along the Vostok ice core is a remarkable indicator of past temperature conditions in central Antarctica⁸. Briefly, high (low) δD corresponds to 'warm' ('cold') temperature. Comparison of this record of relatively large geographical significance⁸ with the CH_4 profile should help in understanding CH_4 cycle/climate interactions. The problem of comparative dating is simplified because both curves are based on the Vostok ice core. The uncertainty that persists because of the evaluation of air-ice difference in age is $\sim 2,000$ years between the two records.

As shown in Fig. 1, there is remarkable similarity between the CH_4 and climate temporal variations, with increasing (decreasing) CH_4 trends when the δD profile indicates warming (cooling). This is also true for the CH_4 oscillation observed between 9 and 12 kyr BP which is probably associated with a cold oscillation corresponding to a cooling of $\sim 2^\circ\text{C}$ at Vostok⁸. The changes of the two parameters are almost simultaneous, going from 'cold' to 'warm' period and *vice versa*. This explains the good correlation ($r^2 = 0.78$) between the two signals which indicates a fundamental link between temperature and CH_4 cycle. Spectral analysis confirms the similarity in the temporal trends of the two records with approximately the same spectra for periodicities corresponding to obliquity and precession (see ref. 17 for δD MTM spectrum). Also cross-spectral analysis (Blackman-Tukey method) between CH_4 data and 'isotope' temperature measurements reveals a coherence of >0.9 for periods >20 kyr, with no significant lag in the phase relationship.

The second salient feature of this comparison lies in some morphological discrepancies in the period between the two interglacials. The δD curve shows for this period (110–20 kyr BP) three minima of temperature at ~ 20 , 60 and 110 kyr BP, and a weak decrease of its baseline. The CH_4 profile exhibits four well marked maxima roughly every 20–30 kyr standing out above a baseline of 400–450 p.p.b.v. and falls to its minimum (350 p.p.b.v.) only during the Last Glacial Maximum. This methane feature probably explains the relatively weak 100-kyr component noted above in the CH_4 spectrum. By comparison, an important component at ~ 100 kyr is found in the δD spectrum¹⁷, indicating the marked glacial-interglacial cycle in the deuterium record.

At this stage of the comparison, we could question how representative, on a global scale, are the temperature amplitude changes recorded in the Vostok core. Indeed, here we are mostly interested in temperature signals that may have affected CH_4 sources, but we are confronted by the sparseness of other continental records covering the same period. Guiot *et al.* recently published a 140-kyr record of temperature based on two pollen records from France²⁰. It reveals that the two periods of relatively warm temperature taking place at the beginning of the last glaciation at ~ 80 and 100 kyr BP led to maximum temperatures close to the interglacial ones, thus showing a similar feature to

the amplitude of CH_4 variations at that time. Second, the large CH_4 decrease (~ 170 p.p.b.v.) at 12–11 kyr BP which parallels a relatively weak cooling ($\approx 2^\circ\text{C}$) at Vostok, is almost synchronous with a large cold stage taking place in the Northern Hemisphere (the Younger Dryas) for which palaeoclimatic investigations reveal, in both Europe and Greenland, a temperature change of $\sim 7^\circ\text{C}$ ^{21,22}. The amplitude relationship between continental temperature at the CH_4 source areas and CH_4 changes could thus have been much closer than the one indicated by the comparison with the Vostok temperature signal.

CH_4 -cycle implications

The Vostok CH_4 profile allows us to investigate the different components of the CH_4 cycle that can have forced the atmospheric CH_4 variations recorded over the full climate cycle.

Atmospheric CH_4 is mainly destroyed by OH oxidation in the troposphere, and a change of palaeolevels of tropospheric OH could therefore have induced the CH_4 variations. The concentration of OH depends on several parameters including levels of reactive species, temperature and moisture (see, for example, ref. 23 for a complete discussion). We previously estimated the glacial-interglacial change of the sink by examining the temperature dependence of the CH_4 -OH reaction rate coefficient and the OH production from H_2O^6 . We concluded that there was an increase of the sink during glacial-interglacial warming that implied a decrease of 20% in the CH_4 concentration. Thus a global source increase by a factor of 2.3 would be required to account for the observed CH_4 increase. This estimate does not take into account possible changes of other reactive species which may have also affected OH levels but we have no way of making reliable estimates for such changes at present.

The most important CH_4 sources are continental and are assumed, excluding anthropogenic influence, to be natural wetlands, termites, methane hydrates and wild animals (see, for example, ref. 24 for a recent estimate). The amount of CH_4

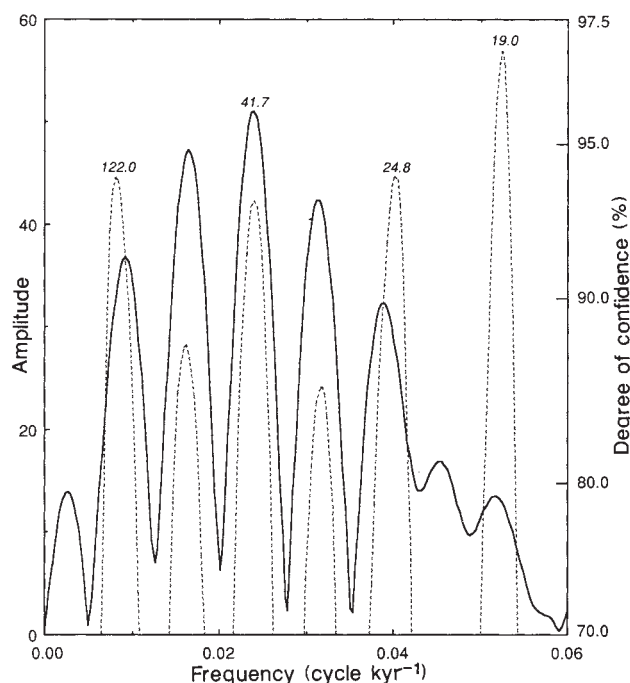


FIG. 2 Spectral analysis of the Vostok CH_4 profile plotted as a function of frequency (kyr^{-1}) and obtained by multi-taper method. The continuous line corresponds to amplitude (left scale) and the dashed line to the degree of confidence obtained from F -test values (right scale). The annotations give the significant periodicities (kyr).

emitted by the wetlands, the principal natural source, depends on their surface and on the flux from each wetland type. Examination of global databases of the present-day surface distribution^{25,26} indicates two principal latitudinal bands, one between 70°N and 50°N and the other between 30°S and 20°N (ref. 25) or 20°S and 10°N (ref. 26). During the glacial-interglacial cycle the high-latitude band could have been controlled by the growth and decay of continental ice sheets inhibiting or liberating some CH₄ sources. Nevertheless the change in continental ice volume estimated from the SPECMAP $\delta^{18}\text{O}$ record¹⁸, which is dominated by a strong 100-kyr cycle¹⁹, suggests that this mechanism was probably weak in controlling past atmospheric CH₄ over the full climate cycle. In fact, because of the slow growth of ice sheets, the high-latitude ecosystems may have moved to middle latitudes. Delcourt and Delcourt²⁷ show, for example, that tundra and boreal forests were widely developed south of the Laurentide ice sheet during the Last Glacial Maximum. On the other hand, during deglaciations, the decay of ice sheets created large areas of open water which may have enhanced the source areas and thus contributed to the approximate doubling of CH₄ at that time.

The low-latitude band may have changed with time because of changes in the hydrological cycle associated with monsoon circulations. Simulations with climate models and palaeodata indicate weaker/stronger monsoon intensity at 18 kyr BP (Last Glacial Maximum)/9–10 kyr BP (for a review see ref. 28). Over the full climate cycle, marine pollen profiles^{29,30} and sapropels (organic-rich layers) in the East Mediterranean Sea^{31,32} indicate four dominant monsoon maxima at ~10 (in two layers for the sapropels), 80, 105 and 120–130 kyr BP. Furthermore, using key time periods to establish a parameterization between orbital parameters, ice-age boundary conditions and monsoon intensity, general circulation models²⁸ simulate qualitatively the four maxima indicated by the proxy data as also shown in Fig. 3. We therefore propose that the monsoon circulation could have had a principal role in controlling palaeomethane concentration through variations of low-latitude wetland areas.

Concerning the wetland fluxes, field measurements indicate an enhanced flux with increasing temperature. We have used this trend to infer that the glacial-interglacial warming impact on the wetland fluxes could explain a large part of the corresponding CH₄ increase⁶. The temperature/CH₄ flux relationship obtained from seasonal observations is, however, probably difficult to apply on a kiloyear timescale because over such a timescale some adaptation of methanogenic bacteria may have occurred in response to lowering temperatures. Such adaptations have been reported in the case of high-latitude flux measurements^{33,34}. The evaluation of the temperature impact on wetland flux and its quantitative application to past conditions therefore require a better understanding of the role of the different parameters involved in controlling CH₄ flux from natural wetlands.

In summary, changes in atmospheric CH₄ measured along the Vostok core over the last climate cycle could be explained by fluctuations in wetland extent, occurring mainly at low latitudes in response to changes of monsoon intensity and, although not well established, by the effect of temperature on CH₄ fluxes evolving from wetlands.

Climate forcing of methane

As discussed above, atmospheric CH₄ levels during the climate cycle seem to be controlled primarily by climate. We now examine how observed CH₄ changes could influence the climate through three processes: direct radiative effect, chemical feedbacks (defined below) and climate feedbacks. Only sparse palaeoclimate data are available for the full glacial-interglacial cycle. Consequently, we restrict our discussion to the role of CH₄ during the transitions from full glacial to interglacial conditions. We should, however, emphasize the remarkable Vostok $\delta\text{D}-\text{CH}_4$ link, which supports the continuous involvement of methane in climate change over the full climate cycle.

We calculated⁶ that the direct radiative effect of CH₄ results in a global surface equilibrium warming of 0.08 °C when CH₄ increased from 350 p.p.b.v. (full glaciation) to 650 p.p.b.v. (interglacial period).

The chemical feedbacks considered are the CH₄-induced modifications through atmospheric chemistry of tropospheric ozone (O₃) and stratospheric water vapour (H₂O), which are two important 'greenhouse' gases. The O₃ feedback depends on the past atmospheric level of NO. The increase in CH₄ leads to a tropospheric O₃ increase in high-NO environments (>10 p.p.t.v., parts per 10¹² by volume)³⁵ and to an unchanged²⁴ or decreasing³⁵ tropospheric O₃ level in the case of low NO contents. In ref. 6 we calculated the effect of a positive O₃ feedback for past conditions and obtained a further surface warming of ~0.05 °C. But the first estimate of NO palaeolevels, based on O₃ measurements made at the end of the last century, indicates that NO concentration was probably low at that time³⁶, suggesting the absence of an O₃ feedback. In the case of stratospheric H₂O feedback, oxidation of one molecule of CH₄ in the stratosphere produces roughly two molecules of H₂O. Through this process, CH₄ accounts for ~55% of the present H₂O level (~6 p.p.m.v.) whereas 45% (2.7 p.p.m.v.) is provided by direct transport from the troposphere to the stratosphere³⁷. This second source of stratospheric H₂O is probably controlled by the low temperature of the tropopause operating as a water-vapour trap. We evaluate the stratospheric H₂O feedback by considering the tropopause temperature to be, as a first approximation, constant with time and the production of stratospheric H₂O by CH₄ oxidation to be constant with elevation. A glacial CH₄ level of ~350 p.p.b.v. would then imply a stratospheric H₂O concentration of 3.4 p.p.m.v. and an interglacial CH₄ value of 650 p.p.b.v. would lead to an H₂O level of 4.0 p.p.m.v. It has been estimated that a doubling of stratospheric H₂O from 3 to 6 p.p.m. (by mass) results in a warming of 0.6 °C at the Earth's surface³⁸.

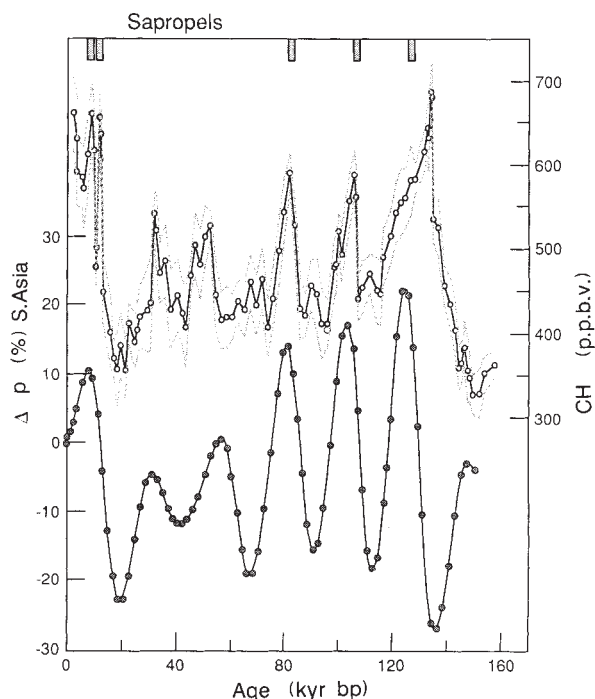


FIG. 3 Upper curve: Vostok CH₄ record plotted against age. Circles correspond to mean values and envelope to the analytical accuracy (2 σ). Lower curve: changes of precipitation (in per cent) over southern Asia simulated by general circulation models²⁸. 0% corresponds to modern boundary conditions. The vertical bars at the top of the figure indicate the presence of sapropels in the East Mediterranean Sea^{31,32}.

Based on this estimate and assuming a linear influence of stratospheric H₂O concentration on temperature, the change of 0.6 p.p.m.v. of stratospheric H₂O calculated above as resulting from glacial-interglacial CH₄ increase would thus imply a warming of 0.07 °C. So the combination of the direct radiative effect of CH₄ and of the stratospheric H₂O feedback would lead to a warming of almost 0.15 °C (and larger if O₃ feedback is positive), that is, ~30% of the direct CO₂ effect for the same transition (~0.5 °C). We may note that in terms of only direct radiative effect (no chemical feedbacks), this percentage drops to 16. We are aware that the above estimate of chemical feedbacks depends on several assumptions that introduce some uncertainty but it does emphasize a potentially important amplification of the direct radiative effect of CH₄ as a result of its chemical activity.

The discussion of the effect of the climate feedbacks driven through the direct radiative effect and the associated chemical feedbacks of the CH₄ change on the glacial-interglacial transition is beyond the scope of this paper. Nevertheless, if we use the estimate of the Goddard Institute for Space Studies three-dimensional global climate model which yields a feedback factor of 3.5 owing to changes in atmospheric water vapour, clouds and snow/ice cover³⁹, the glacial-interglacial CH₄ climate forcing including these feedbacks would amount to ~0.5 °C. These climate feedbacks would also apply to CO₂ direct forcing and would lead to a CO₂-induced surface warming of 1.75 °C. The mean global surface warming corresponding to the last deglaciation can be estimated to be 4.5 ± 1 °C (ref. 40). The combined climate forcing of CO₂ and CH₄ (~2.3 °C) can therefore explain ~50% (~10% for CH₄ alone) of the glacial-interglacial warming, confirming our previous conclusion⁶.

Younger Dryas CH₄ oscillation

Palaeoclimate data reveal a large cooling, the Younger Dryas, ~11–10 kyr BP in the Northern Hemisphere, particularly in Europe and Greenland. This cooling follows the warming of Bölling-Alleröd interstadial (13–12 kyr BP)^{21,22}. In the Southern Hemisphere, Antarctic ice cores show a cooling of weak amplitude during the last deglaciation^{8,41,42} but its relationship with the northern event is not firmly established. In fact, the CH₄ oscillation (~170 p.p.b.v.) recorded at Vostok around the period

of concern, which represents ~60% of the full glacial-interglacial variation (300 p.p.b.v.), is the first large-amplitude signal recorded in the Southern Hemisphere that is of global significance.

The cooling of North Atlantic sea surface temperature is suspected to have triggered the Younger Dryas^{21,22}. The CH₄ oscillation at Vostok could be linked to the ocean cooling through two processes: (1) an abrupt atmospheric cooling of several degrees (~7 °C) at high latitudes^{21,22} which may have forced the freezing of bogs in these regions; (2) a decrease in precipitation rates at low latitudes³² which may have influenced the wetland areas at these latitudes.

If the assumptions made above about the feedbacks associated with CH₄ change also apply to the Younger Dryas, the CH₄ decrease at 12–11 kyr BP would have led to a mean cooling of the Earth's surface of ~0.3 °C (including chemical and climate feedbacks).

Further CH₄ analyses of this period both on Greenland and Antarctic ice cores should provide useful information for linking observations in the Northern and Southern Hemispheres and for understanding the coupling between such abrupt climate and atmospheric CH₄ changes.

Conclusions

The record of atmospheric CH₄ over the last climate cycle obtained from the Vostok ice cores reveals fluctuations of natural CH₄ level in the 350–650 p.p.b.v. range. The natural behaviour of CH₄ could be mainly the result of the climate impact on the CH₄ cycle and the role of the low latitudes may be important. The impact of CH₄ (including chemical feedback) on climate is estimated to be ~0.15 °C for glacial-interglacial transitions, that is, ~30% of the CO₂ radiative effect occurring at the same time. Apart from the long-term trend, the Vostok CH₄ profile indicates that methane could also react to abrupt climate change as suggested by the large-amplitude CH₄ oscillation occurring in the last deglaciation which is probably associated with the Younger Dryas climate event.

The fundamental link between CH₄ and climate variations indicated by the Vostok record suggests that the natural CH₄ cycle may provide a positive feedback in any future global warming. □

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Inhibition of mutant troponin C activity by an intra-domain disulphide bond

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Triggering of contraction in striated muscles involves a conformational transition in the N-terminal domain of troponin C, the calcium-binding component of thin filaments. We have designed a mutant troponin C in which the key conformational transition and the calcium-regulatory activity are reversibly blocked by the formation of a disulphide bridge. Our results may be applicable to other proteins of the same family of calcium-binding proteins.

CALCIUM-induced changes in the conformation of troponin C (TnC), the calcium-binding component of thin filaments in skeletal and cardiac muscles, are transmitted to other components of the thin filament: troponin I (TnI), troponin T (TnT), tropomyosin and actin, triggering muscle contraction^{1,2}. The three-dimensional crystal structure of TnC isolated from turkey and chicken skeletal muscle has been solved and refined recently to better than 0.2-nm resolution^{3,4}. The molecule seems to have two globular domains each containing a pair of Ca²⁺-binding sites, each in turn consisting of a 13-residue loop flanked by two α -helices. Sites I and II in the N-terminal domain are the 'triggering' low-affinity Ca²⁺-specific sites, and sites III and IV in the C-terminal domain are the high-affinity Ca²⁺-Mg²⁺ sites believed to be always saturated with Ca²⁺ or Mg²⁺ in the living muscle, possibly providing structural stability to the molecule^{1,2}. The helices are labelled A-H starting at the N-terminus. Helix D, the D-E linker and helix E form a continuous central α -helix connecting the two domains. The structure of crystalline TnC corresponds to the inhibited form of this protein as it contains Ca²⁺ only in sites III and IV. A growing body of evidence derived from fluorescence energy transfer⁵⁻⁷, low-angle X-ray scattering⁸ and chemical crosslinking⁹ suggest that the molecule is more compact when in solution at neutral pH and in the complex with TnI.

The nature of the triggering conformational transition in TnC is unclear. Although sites I and II in the N-terminal domain are believed to be directly involved¹⁰, fragments derived from either of the two halves of the molecule have partial regulatory activity provided helix E is present¹¹. Despite the lack of direct interaction between the two halves of TnC, there is some inter-domain communication, especially in the complex with TnI, suggesting that both domains may be involved in activation^{12,13}. Also it was suggested that the stability of the central helix may be important for TnC function¹⁴, but the substitution of Ala or Pro for Gly in the central helix does not affect the biological activity of TnC (ref. 15), and deletion of the tripeptide Lys-Gly-Lys in the same segment of the central helix has only a slight effect¹⁶. These results virtually exclude active involvement of the central helix in the Ca²⁺-induced triggering transition in TnC.

The helices forming the Ca²⁺-binding units in the N-terminal Ca²⁺-free domain of the crystalline TnC are almost antiparallel to each other, unlike the perpendicular orientation of homologous segments in the C-terminal domain of TnC and in

all other Ca²⁺-filled Ca²⁺-binding sites whose structure is known^{17,18}. Herzberg *et al.*¹⁷ proposed that calcium binding to sites I and II causes a change in the relative disposition of the helices to resemble that found in the Ca²⁺-filled sites III and IV. Such a transition would result in partial exposure of some hydrophobic side chains, providing an additional interaction site for TnI. The Ca²⁺-induced increase in accessibility of Cys-84 in cardiac TnC (refs 19, 20) and Cys-57 in a mutant TnC (ref. 46) seems to be consistent with this model. Here, we describe a TnC mutant in which the putative movement of the α -helices is prevented by a disulphide bridge. This leads to altered Ca²⁺-binding properties and loss of regulatory activity.

Design of a mutant TnC

Herzberg *et al.*¹⁷ suggest that the relative position of helix B with respect to helix C does not change, but that both move away from helices A and D when Ca²⁺ binds. Movement could be prevented by a disulphide bridge between cysteine residues substituted for amino acids whose side chains are close to each other in the Ca²⁺-free form of TnC but undergo a large increase in distance on Ca²⁺ binding. The best candidates for mutation seemed to be Gln-51 and Asp-89 in turkey skeletal TnC whose distance, according to the model, increases by ~1.4 nm on Ca²⁺ binding at sites I and II (ref. 17). But calculations based on the X-ray coordinates indicated that on replacement of the two residues with Cys the closest possible distance between the sulphur atoms would still be >0.4 nm. If Gln 85, a residue one

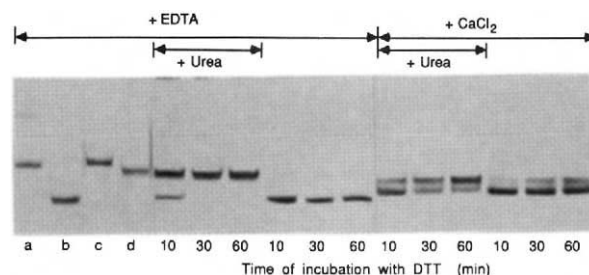


FIG. 1 Reduction of the intramolecular disulphide bond in TnC4882. Electrophoretic mobilities of sTnC (lanes a, c) and of TnC4882 (lanes b, d) are compared before (a, b) and after (c, d) treatment with DTT. Electrophoresis was run on 10% polyacrylamide gel containing 6 M urea and 80 mM glycine-tris, pH 8.6 (also used as electrophoresis buffer). The gel and the electrophoresis buffer contained either 1 mM EDTA or 1 mM CaCl₂ as indicated. The label (+Urea) denotes samples that had been treated with DTT in the presence of 6 M urea. Labels (+EDTA) and (+CaCl₂) refer to both the conditions of incubation with DTT and the conditions of electrophoresis. METHODS. To obtain TnC4882, *in vitro* site-directed mutagenesis was carried out using the Muta-Gene M13 kit (Bio-Rad) following the method of Kunkel⁴³, on full-length rabbit skeletal TnC complementary DNA (ref. 44) subcloned into M13mp18. Over-expression was achieved using the plasmid pKP1500 as expression vector and *Escherichia coli* strain KP3998 as host⁴⁵. The TGC codon coding for Cys 98 was first mutagenized to the CTT codon for Leu (ref. 46). The CAG codons of Gln 48 and Gln 82 were then mutagenized simultaneously to the TGC codon for Cys. Mutations were selected and confirmed by dideoxynucleotide sequencing⁴⁷. Expression, isolation and purification of mutant TnC was according to Wang *et al.*⁴⁶.

TABLE 1 TnC binding constants

	EGTA		MgCl ₂		CaCl ₂	
	F_i/F_0	K	F_i/F_0	K	F_i/F_0	K
sTnC	2.6	3.3×10^6	2.0	3.6×10^6	0.81	1.8×10^8
ox-TnC4882	3.2	1.1×10^6	1.6	1.5×10^6	1.13	1.2×10^7
CM-TnC4882	1.9	2.5×10^6	1.9	2.3×10^6	0.86	1.1×10^8

TnC labelled with 1,8-IAEDANS at Cys-133 (0.5 μ M) was used for titrations in the presence of Ca²⁺. For all other titrations TnC samples were labelled with dansylaziridine. TnC concentrations were 0.5–1.5 μ M. Titrations were carried out in a solution containing 0.1 M KCl, 20 mM HEPES pH 7.5, 0.5 mM EGTA and 10 mM MgCl₂ or 1 mM CaCl₂ as indicated. In all cases the unlabelled component was the titrant. Binding constants were obtained from titration data by applying an iterative nonlinear least-square fitting procedure³⁸. Averages from two titrations of the fitted values of K and F_i/F_0 are given, where K , F_i and F_0 are: equilibrium binding constant, fluorescence at saturation and the fluorescence in the absence of the second component, respectively. Labelling of TnC with 1,8-IAEDANS was based on the finding that Cys-133 is accessible in the troponin complex³⁹. Lyophilized troponin was dissolved in a solution containing 6 M urea, 0.1 M KCl 25 mM HEPES pH 7.5 and 5 mM DTT. After overnight dialysis against a solution containing 0.1 M KCl, 25 mM HEPES, pH 7.5 and 0.1 mM CaCl₂ solid 1,8-IAEDANS was added in 10-fold molar excess over total SH content and the solution was incubated in the dark for 4 h at 25 °C. After addition of DTT (final concentration 10 mM) the sample was dialysed overnight against a solution containing 6 M urea, 25 mM HEPES pH 7.5, 1 mM EDTA and 2 mM DTT. The troponin subunits were separated on a Sephadex DEAE A-50 column with a linear gradient of 0–0.6 M KCl. The extent of labelling was calculated from the protein concentration measured with the Bradford (Bio-Rad) reagent⁴⁰ and from the label concentration based on the extinction coefficient $\epsilon = 6,000 \text{ M}^{-1} \text{ cm}^{-1}$ (345 nm).

helical turn closer to the N terminus, rather than Asp 89, was replaced by Cys the two sulphur atoms could be brought into contact by rotation about the Cys C α –C β bonds without any adjustment to the polypeptide backbone. We therefore substituted Cys for Gln 48 in the B–C linker and for Gln 82 in the D segment of the central helix in rabbit skeletal TnC (sTnC), the residues homologous to Gln 51 and Gln 85, respectively, in turkey skeletal TnC. To avoid the unlikely possibility that one of the two newly introduced cysteines would form a disulphide bridge with Cys 98, the single cysteine residue of sTnC, we have replaced it with Leu—the residue found at that position in bovine cardiac TnC. Two additional differences between the mutant (TnC4882) and sTnC are the presence of Met at the N terminus and the lack of acetylation of the N-terminal α -NH₂, which does not affect the properties of TnC (refs 15, 16).

Reactivity of thiol groups in mutant TnC

Freshly prepared TnC4882 gave no reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) indicating that the two cysteines had formed a disulphide bridge. Further evidence for intramolecular disulphide formation was obtained from electrophoresis in a urea-EDTA polyacrylamide gel. Without treatment with dithiothreitol (DTT) the mobility of TnC4882 was much higher than that of sTnC (Fig. 1), but after incubation with 10 mM DTT in the presence of 5 M urea and 1 mM EDTA its mobility was nearly the same as that of sTnC. The unusually high electrophoretic mobility of the oxidized form of the mutant TnC (ox-TnC4882) is presumably caused by the intramolecular disulphide bond stabilizing the protein against unfolding by urea.

The difference in migration velocity between ox-TnC4882 and the reduced form enabled us to monitor its reduction. We incubated ox-TnC4882 with 10 mM DTT in various conditions and analysed the products on urea-polyacrylamide gel (Fig. 1). The disulphide bridge in ox-TnC4882 is indeed very stable as essentially no reduction occurred in 1 h incubation in the presence of EDTA. The same treatment in the presence of Ca²⁺ yielded only partial reduction resulting in the formation of a slower migrating band. Thus the disulphide bond is essentially inaccessible in the absence of Ca²⁺ and becomes only partially accessible on Ca²⁺ binding. A fully reduced protein can be obtained by the combined action of DTT, 6 M urea and EDTA (Fig. 1). In subsequent experiments we have compared proper-

ties of ox-TnC4882 with those of the carboxamidomethylated form of the mutant TnC (CM-TnC4882) obtained by treatment with iodoacetamide after reduction in the unfolded state.

Ca²⁺ binding properties of TnC4882

We used two different spectroscopic probes to monitor Ca²⁺ binding to the two classes of Ca²⁺-binding sites in the mutant TnC. To monitor Ca²⁺ binding sites I and II the proteins were labelled with dansylaziridine—a fluorescent probe that binds covalently to Met 25 in sTnC (ref. 21). The transition midpoint for CM-TnC4882 coincides with that obtained for sTnC; the transition for ox-TnC4882 is shifted by 0.6 pCa unit towards higher Ca²⁺ concentrations (Fig. 2). Thus by preventing separation between residues 48 and 82, both of which are some 2 nm away from the Ca²⁺-binding loops, we were able to decrease

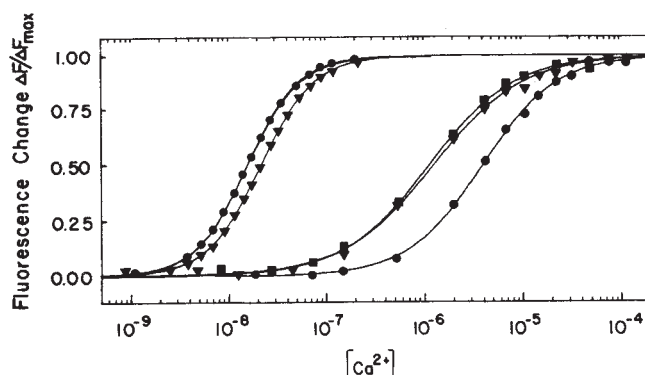


FIG. 2 Calcium-binding properties of mutant TnC. Two sets of experiments illustrate Ca²⁺ binding to the two classes of sites in sTnC (▼), ox-TnC4882 (●) and CM-TnC4882 (■). Binding of Ca²⁺ to the high-affinity sites was monitored by tyrosine fluorescence²² (λ_{exc} 280 nm, λ_{em} 308 nm) and the binding to the low affinity sites by dansylaziridine²¹ (DANZ) fluorescence (λ_{exc} 340, λ_{em} 520 nm). With ox-TnC4882 there was a substantial decrease in DANZ fluorescence associated with Ca²⁺ binding to the high-affinity sites followed by an increase in fluorescence. Only the latter portion of the curve was fitted. The difference between data points for the high-affinity binding in ox-TnC4882 and CM-TnC4882 are smaller than the size of symbols; one type of symbol (●) represents both sets of data. Averaged values of the fitting parameters obtained from two titrations are shown below.

	Low-affinity sites			High-affinity sites		
	$10^{-6} \times K$	n	$p\text{Ca}_{1/2}$	$10^{-12} \times K$	n	$p\text{Ca}_{1/2}$
sTnC	1.26	1.00	5.90	0.40	1.51	7.68
ox-TnC4882	1.07	1.12	5.32	3.02	1.60	7.80
CM-TnC4882	1.26	1.02	5.98	2.52	1.59	7.80

METHODS. Calcium titrations were carried out in a solution containing 0.1 M KCl, 0.1 M HEPES pH 7.5 and 2 mM (ethylene-bis(oxyethylenetriolo)) tetraacetic acid (EGTA) or 1 mM EGTA + 1 mM nitrilotriacetic acid (NTA), for binding to high-affinity sites and low-affinity sites respectively. At pH 7.5 the EGTA-NTA system provides good Ca²⁺-buffering down to $p\text{Ca} = 4.0$ (ref. 12). Concentrations of free Ca²⁺ were calculated according to Perrin and Sayce⁴⁸ using published values for the binding constants⁴⁹. The fluorescence data were fitted with the equation: $F = F_0 + F_1 K [\text{Ca}]^n / (1 + K [\text{Ca}]^n)$, where F_0 , F_1 , K and n are the initial fluorescence, maximal fluorescence change, cumulative binding constant and the Hill coefficient, respectively. The relation between the transition midpoint $p\text{Ca}_{1/2}$, often used to characterize Ca²⁺ affinity, and K and n is $p\text{Ca}_{1/2} = (\log K)/n$. Labelling of TnC with dansylaziridine was as in Johnson *et al.*²¹. The labelling yield was significantly lower in the case of ox-TnC4882 (20–30%) than in the case of sTnC and CM-TnC4882 (80–95%). For carboxamidomethylation of TnC4882 lyophilized protein was dissolved in a solution containing 6 M urea, 0.1 M KCl, 0.2 M Tris pH 8.0, 1 mM EDTA and 20 mM DTT and incubated for 1 h at 25 °C. The sample was protected from light with aluminium foil, placed in an ice bath and solid iodoacetamide was added in a 1:1 molar ratio to the total SH content. After 20 min incubation in the dark at 0 °C, DTT was added (20 mM final concentration) and the sample was dialysed against a solution containing 0.1 M KCl, 20 mM HEPES, pH 7.5 and 2 mM EDTA.

TABLE 2 Regulation of myofibrillar ATPase by mutant TnC

	Concentration of TnC (μ M)	ATPase activity ($\text{nmol P}_i \text{ mg}^{-1} \text{ min}^{-1}$)		Relative activity (%)
		+Ca ²⁺	-Ca ²⁺	
No addition		74.4 $\pm$ 1.7 (12)	47.7 $\pm$ 1.8 (10)	
+sTnC	0.5	282 $\pm$ 11 (5)	85.4 $\pm$ 9.2 (3)	100
	2.0	304 $\pm$ 13 (8)	71.9 $\pm$ 3.2 (6)	100
+ox-TnC4882	0.5	117 $\pm$ 6.8 (7)	82.7 $\pm$ 7.6 (7)	20
	2.0	118 $\pm$ 7.2 (7)	71.0 $\pm$ 2.1 (6)	19
+CM-TnC4882	0.5	271 $\pm$ 11 (6)	76.9 $\pm$ 7.1 (5)	99
	2.0	264 $\pm$ 12 (8)	73.2 $\pm$ 2.9 (5)	83

Means $\pm$ s.e.m. and number of measurements (in parentheses) are given. The regulatory activity of mutant TnC is expressed as the ratio of the increase in ATPase activity in the presence of Ca²⁺ induced by the addition of TnC mutant to the increase induced by the addition of rabbit skeletal TnC, multiplied by 100. Myofibrils were prepared by eight washings by centrifugation and resuspension of fresh homogenized rabbit skeletal muscles with a solution containing 80 mM KCl, 1 mM TES pH 7.0, 0.1 mM DTT and stored in 50% glycerol at -20 °C. The method of extracting TnC by washing with low ionic strength solution containing EDTA⁴¹ was improved by additional treatment with TnI. Glycinated myofibrils (2.4 ml, 1.3 mg ml⁻¹) were washed twice in 100 volumes of a solution containing 5 mM EDTA, 10 mM imidazole pH 7.0, 10 mM 2-mercaptoethanol, 2 μ M phenylmethylsulphonyl fluoride (PMSF) and 1% Triton X-100; 5 min incubation was followed by 15 min centrifugation at 1500g. Myofibrils were suspended in 3 ml of a solution containing 0.4 M KCl, 0.1 mM EDTA, 10 mM imidazole pH 7.0, 10 mM 2-mercaptoethanol, 2 μ M PMSF, 1% Triton X-100 (high salt buffer) and mixed with 0.5 ml of a solution containing 7.5 mg of TnI in 0.4 M KCl, 10 mM imidazole pH 7.0, and 10 mM 2-mercaptoethanol. The suspension was incubated for 15 min and myofibrils were collected by centrifugation and washed twice with 5 volumes of the high-salt buffer. Finally myofibrils were washed twice with 5 volumes of a solution containing 90 mM KCl, 10 mM imidazole pH 7.0, and 1% Triton X-100, and suspended in 3 ml of the same solution without Triton X-100. ATPase activity was measured from the amount of phosphate liberated from ATP measured colorimetrically⁴². sTnC or mutant TnC was added to 0.5 or 2 μ M final concentration to a 0.25 ml sample of TnC-depleted myofibrils (0.5 mg ml⁻¹) in a solution containing 90 mM KCl, 10 mM imidazole pH 7.0, 2 mM MgCl₂ and either 0.2 mM CaCl₂ or 1 mM EGTA. The reaction was initiated by addition of 1 mM ATP and terminated after 5 min by addition of 0.25 ml of 2% SDS.

substantially the affinity for Ca²⁺, consistent with the proposal that a specific geometry of the helix-loop-helix unit is required for optimal Ca²⁺ binding. These results also suggest a considerable flexibility in the structure, enabling Ca²⁺ to bind even if the whole unit cannot assume the conformation induced by Ca²⁺ in the native protein.

Binding at the high-affinity sites III and IV was monitored by changes in tyrosine fluorescence²². Titration curves of ox-TnC4882 and CM-TnC4882 were indistinguishable; affinity was slightly higher than that of sTnC (Fig. 2). The difference is most probably a result of higher stability of the Ca²⁺-bound conformation of the C-terminal domain resulting from substitution of Leu for Cys 98. All three proteins exhibit some cooperativity in binding to the high-affinity sites, the Hill coefficient being 1.5–1.6. Binding to the low-affinity sites showed no cooperativity.

Interaction of TnC4882 with TnI and TnT

An important characteristic of TnC is its ability to form a complex with TnI and TnT. Electrophoresis on urea-polyacrylamide gel indicates that both ox-TnC4882 and CM-TnC4882 retain this ability (Fig. 3). To obtain a quantitative measure of the strength of the interaction with TnI we have used an equilibrium titration method. Binding in the presence of Ca²⁺ was monitored by the fluorescent probe 8-(iodoacetamidoethyl) aminonaphthalene-1-sulphonic acid (1,8-IAEDANS) attached to Cys 133 in TnI. For titrations in the absence of Ca²⁺, sTnC and the two forms of TnC4882 were labelled with dansyl-aziridine²¹. In the absence of Ca²⁺ the affinity of ox-TnC4882 for TnI is only slightly lower than that of sTnC or CM-TnC4882, but in the presence of Ca²⁺ the decrease in affinity is 15-fold (Table 1). A likely interpretation of these results is that the disulphide bond in ox-TnC4882 prevents the opening of a TnI-binding site even though Ca²⁺ ions are bound to the triggering sites.

Regulatory activity

The importance of Ca²⁺-induced conformational change in the N-terminal domain of TnC is clearly shown by experiments designed to test the regulatory activity of the mutant TnC. For these we used myofibrils from which the indigenous TnC had been removed, thereby rendering the ATPase insensitive to Ca²⁺. Although addition of sTnC or CM-TnC4882 restored the Ca²⁺ sensitivity of the ATPase, ox-TnC4882 had virtually no effect even if its concentration was increased fourfold (Table 2). High-speed sedimentation showed that ox-TnC4882 was bound to reconstituted thin filaments whether Ca²⁺ was present or not (data not shown) indicating that the lack of activity of ox-TnC4882 did not result from its inability to bind to the thin filament but from impaired regulatory properties. The recovery of activity on reduction and carboxamidomethylation provides unequivocal evidence that the intramolecular disulphide was solely responsible for the impaired properties.

Discussion

We show here the modification of several properties of a protein—including interaction with a divalent cation, protein-protein interaction and biological activity—by blocking a transition between two conformations. Modification of protein structure by introducing a disulphide link by genetic engineering has recently been used for studies of lysozyme²³ and calmodulin²⁴, yielding important insights into structure-function relations. In both studies the disulphide bond bridged two separate domains. We show that a similar approach can be used to study conformational transitions within the same domain. Our data have important implications for both the mechanism of Ca²⁺-signal transduction in troponin and the mechanism of Ca²⁺-binding to the EF-hand type Ca²⁺-binding proteins.

Introduction of a disulphide bridge between cysteine residues located in the segment connecting helix B with helix C and the central helix, respectively, in the N-terminal domain of sTnC virtually abolishes its regulatory activity. Activity can be fully restored by reduction of the disulphide bridge, providing evidence that Ca²⁺ binding to sites I and II causes separation between the B-C linker and the central helix. This agrees with the proposed conformational change in TnC (ref. 17) and with this transition being an essential step in activation.

Owing to the location of Cys 48 in the B-C linker, the disulphide link in ox-TnC4882 most probably restricts adjustment of the interhelical angles in both site I and site II. Recent investigations of the regulatory activity of mutants of cardiac TnC in which either site I or site II or both sites in the N-terminal domain can bind Ca²⁺ indicated that site II was required to regulate actomyosin ATPase²⁵. It seems likely that in skeletal TnC also site II is responsible for the regulatory activity and

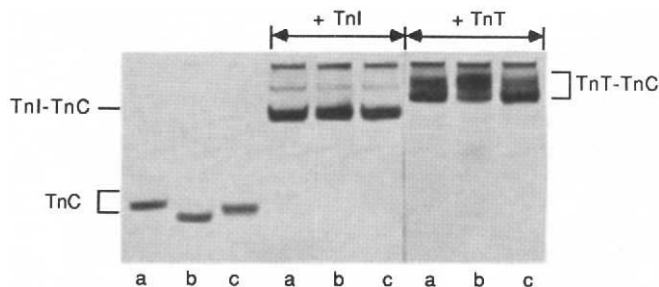


FIG. 3 Complex formation between TnC4882 and TnI and TnT. sTnC (lane a), ox-TnC4882 (lane b) and CM-TnC4882 (carboxamidomethylated TnC4882) (lane c) were run alone and with twofold molar excess of rabbit skeletal TnI or TnT as indicated. Electrophoresis was carried out on urea-polyacrylamide gel in the presence of 1 mM CaCl₂. In the electrophoresis conditions TnI and TnT do not enter the gel unless they are complexed with TnC. Tn components TnI and TnT were obtained as in ref. 50.

that the lack of activity of ox-TnC4882 results from failure to open a cavity between helix C and helix D on Ca^{2+} binding to site II.

Although the present data leave little doubt about the nature of the triggering transition in the N-terminal domain in TnC it should be emphasized that this transition may be only a part of the regulatory mechanism as the TnC fragment containing only the N-terminal domain is unable to confer Ca^{2+} sensitivity on reconstituted actomyosin¹¹. Several lines of evidence point to a rather extensive interface between TnC and TnI. Peptide studies, crosslinking, and ¹H-NMR indicate that the segment comprising residues 89–100 in the C-terminal domain of TnC interacts with the segment of TnI capable of inhibiting actomyosin ATPase (residues 96–116) (refs 26–28). Two-step zero-length crosslinking⁹ has recently provided evidence that helix C in TnC (residues 51–62) also interacts with segment 96–116 in TnI raising the possibility that both domains of TnC interact with the same short segment of TnI. If this is the case the TnC-TnI complex should have a compact structure similar to that recently proposed for the complex of calmodulin with the fragment of myosin light chain kinase^{24,29}.

The nature of the interactions at the TnC-TnI interface is still not clear. Fragments of TnI able to bind TnC contain a number of positively charged Lys and Arg residues whose ¹H-NMR signals are altered on interaction with TnC (refs 26, 27, 30). Peptide studies implicated Glu-rich segments of TnC in the interaction with TnI (ref. 11). Zero-length crosslinking induced by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) between ϵ -NH₂ groups of Lys and COOH groups of Glu and Asp is an efficient way to crosslink TnC with TnI (ref. 9). These observations suggest that charge-charge type interactions have an important role in the formation of the TnC-TnI complex. On the other hand the triggering transition clearly leads to the exposure of some hydrophobic side chains that in the resting state form a contact between helix C and the central helix^{17–20}. Thus it seems likely that both charge-charge and hydrophobic interactions are involved in the TnC-TnI binding or, perhaps, each type of interaction has a specific function at different steps of the activation process.

TnC is one of the best known examples of the family of homologous Ca^{2+} -binding proteins with a common motif for

Ca^{2+} binding—the helix-loop-helix structure first described for the soluble Ca^{2+} -binding protein parvalbumin³¹. There have been several attempts to correlate the composition of the binding site with the affinity and specificity for calcium^{32–34}. The most promising prediction of Ca^{2+} affinity was achieved when the sequence of the whole helix-loop-helix unit was considered³³. In our experiments none of the ligands involved in Ca^{2+} chelation was changed, nor were amino acids in the vicinity of the cation-binding site replaced, yet the affinity for Ca^{2+} was decreased on blocking a conformational transition some 2 nm away from the binding site. These results clearly demonstrate the importance for Ca^{2+} affinity of the overall folding energy of the two-site domain.

Are conclusions about conformational transitions in TnC applicable to other Ca^{2+} -binding proteins of the EF-hand family? The fact that calmodulin can bind TnI and can replace TnC in the activation of regulated actomyosin strongly indicates that at least some part of the activatory mechanism is common to both proteins. An essential feature of the mechanism proposed by Herzberg *et al.*¹⁷ and experimentally confirmed here is the opening of a hydrophobic pocket between two helices flanking a Ca^{2+} -binding loop. The Ca^{2+} -dependent formation of hydrophobic sites in calmodulin and TnC was well characterized before their three-dimensional structure became known³⁵. Using this criterion, a number of Ca^{2+} -binding proteins have been discovered on hydrophobic chromatography on phenyl-sepharose which indicates Ca^{2+} -dependent exposure of similar hydrophobic sites in these proteins. At present more than 160 Ca^{2+} -binding proteins are known, all using the helix-loop-helix motif, and having remarkable sequence homologies^{33,36,37}. If protein segments of similar sequence perform similar functions, that is binding Ca^{2+} and transmitting a Ca^{2+} -stimulatory message to other proteins, then it seems reasonable to assume that they operate in a similar way.

It is likely that the conformational transition involving the separation of helical segments in a Ca^{2+} -binding unit of the type occurring in the N-terminal domain in TnC is not unique to this protein, but represents a universal mechanism by which Ca^{2+} -binding proteins transmit the regulatory Ca^{2+} signal to their targets. □

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Collimation of electromagnetic radiation in optically thick astrophysical synchrotron sources

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COLLIMATION of energy output into two oppositely directed beams seems to be very common in the Universe, particularly in extra-galactic radio sources, but also within the Galaxy, for example SS433. The collimation mechanism is still in doubt. Here I discuss the possibility of collimation arising from anisotropic synchrotron absorption whereby electromagnetic waves are less strongly absorbed when propagating parallel, rather than perpendicular, to a magnetic field. This discussion can apply to any object which is optically thick to synchrotron emission, but I particularly have in mind the collimation of infrared/optical/ultraviolet radiation in active galactic nuclei. The beamed radiation may be seen in blazars¹ and in the recent observations by di Serego Alighieri *et al.*². The beams may also drive double radio sources.

Electromagnetic radiation passing through a plasma containing energetic cosmic-ray electrons and a magnetic field is absorbed by inverse synchrotron absorption. For isotropic electrons with a power-law energy distribution $n(E) = KE^{-a}$, Melrose³ gives an absorption rate (in c.g.s. units)

$$\gamma = \frac{L\pi}{\sqrt{3}} \omega K (mc^2)^{2-a} \left(\frac{e}{mc\omega} \right)^2 \left(\frac{3\Omega \sin \theta}{2\omega} \right)^{(1+a/2)} \quad (1)$$

where ω is the frequency of the electromagnetic radiation, Ω is the non-relativistic electron gyrofrequency, m is the mass of the electron, e is the charge of the electron, c is the speed of light and θ is the angle between the magnetic field and the direction of electromagnetic propagation. L is a number of the order of one which depends on polarization and on a . As $\gamma \propto \sin \theta^{(1+a/2)}$, and a is typically between 2 and 3, absorption is strong when radiation crosses a magnetic field and weak when it propagates along it. Suppose that a radiation source, driven by a compact object, lies at the centre of the plasma. Radiation is able to escape relatively freely where the magnetic field is directed radially away from the source, but is more strongly absorbed in directions where the magnetic field is at an angle to the radial direction. This might result in radiation preferentially escaping in directions where the magnetic field is radial to the source, that is, the emission of radiation might be collimated. The division of the plasma into an inner emitting region and an outer absorbing region is a simplification because the electrons in the outer region will emit, but the model is sustainable if the effective electron temperature is higher in the centre.

To assess the strength of this effect, consider the case of a plasma supporting a dipolar field

$$\mathbf{B} = \mu/r^3 - 3(\mu \cdot \mathbf{r})\mathbf{r}/r^5 \quad (2)$$

where μ is the magnetic dipole moment. I assume that the plasma is uniformly permeated by energetic electrons with a power-law distribution $n(E) = KE^{-2}$. Because the magnetic field decreases as r^{-3} , the plasma is optically thick near the centre, and optically thin at large r . The boundary of the optically thick region acts as a photosphere. But as the absorption is anisotropic, the position of the optically thick boundary depends on the direction of emission, or equivalently on the direction from which the plasma is viewed. The optical depth, $\tau(\mathbf{r}, \mathbf{n}) = \int (\gamma/c) ds$ (integrated along the ray path), for radiation escaping to infinity from a point $\mathbf{r}$ in a direction defined by a unit vector $\mathbf{n}$, can be calculated analytically (assuming that L is constant along the

ray path) for this case

$$\begin{aligned} \tau = & -\frac{1}{16} \left(\frac{R_0}{r} \right)^5 \left\{ \left(16 - \frac{(\mu \cdot \mathbf{n})^2}{\mu^2} + 25 \frac{(\mu \cdot \mathbf{b})^2}{\mu^2 b^2} \right) \right. \\ & \times \left(\frac{2 \sin^3 \varphi \cos \varphi + 3 \sin \varphi \cos \varphi - 3 \varphi}{8 \sin^5 \varphi} \right) \\ & + \left[\left(3 - 18 \frac{b^2}{r^2} \right) \frac{(\mu \cdot \mathbf{n})^2}{\mu^2} + \left(5 + 18 \frac{b^2}{r^2} \right) \frac{(\mu \cdot \mathbf{b})^2}{\mu^2 b^2} \right] \cos \varphi \\ & \left. + \left(16 - 36 \frac{b^2}{r^2} \right) \left(\frac{(\mu \cdot \mathbf{b})(\mu \cdot \mathbf{n})}{\mu^2 r} \right) \right\} \end{aligned} \quad (3)$$

where $\mathbf{b} = \mathbf{r} - (\mathbf{r} \cdot \mathbf{n})\mathbf{n}$, $\cos \varphi = (\mathbf{r} \cdot \mathbf{n})/r$, $0 \leq \varphi \leq \pi$ and the constant R_0 is

$$R_0^5 = \frac{9\pi L \mu^2 \omega}{4\sqrt{3}} K \left(\frac{e}{mc\omega} \right)^4 \quad (4)$$

The value of R_0 can be made more intelligible by choosing a characteristic radius R_c (as yet undefined) and defining B_c as the magnetic field at that radius on the magnetic equator, in which case $\mu = B_c R_c^3$. Also, let $\Omega_c = eB_c/mc$ be the non-relativistic gyrofrequency and $U_B = B_c^2/8\pi$ the magnetic energy density at that point. If the electron energy spectrum extends over a factor of 10^3 in energy, then the energetic electron density is $U_e = 3K \ln 10$. This gives

$$R_0^5 = (\omega/c)(\sqrt{3}L/32 \ln 10)(U_e/U_B)(\Omega_c/\omega)^4 R_c^6 \quad (5)$$

or, equivalently

$$\left(\frac{R_0}{pc} \right)^5 = 104 \left(\frac{L}{3} \right) \left(\frac{U_e}{U_B} \right) \left(\frac{B_c}{G} \right)^4 \left(\frac{\lambda_s}{cm} \right)^3 \left(\frac{R_c}{pc} \right)^6 \quad (6)$$

where λ_s is the wavelength of the synchrotron emission. The correct value³ for L when $a=2$ and the polarization electric vector is orthogonal to B is $L=5.6$. In the other linear polarization, $L=0.8$.

Setting $\tau=1$ gives the position of the photosphere when viewed from a direction $\mathbf{n}$. It would be possible to plot the photosphere for a selection of directions $\mathbf{n}$. Instead, I find the minimum value of τ with respect to $\mathbf{n}$ at any $\mathbf{r}$ and define the photosphere as the surface on which this minimum value is $\tau=1$. The photosphere so defined is plotted in Fig. 1. The assumption that L is constant is valid for the ray paths with minimum τ .

Figure 1 shows that the photosphere penetrates to the centre of the plasma along the magnetic poles. If the primary energy source lies at the centre of the plasma, energizing the plasma out to some radius less than the maximum radius of the photosphere, then radiation is able to escape near the poles, but not near the equator. From the shape of the photosphere it is clear that the escaping radiation will be collimated to a greater or lesser degree depending on the extent of the emission region. The narrow funnel over the pole suggests that a beam divergence as low as a few degrees may be possible.

The radius of the photosphere on the equator is

$$\begin{aligned} R_e = 0.72 R_0 = & \left(\frac{L}{3} \right)^{1/5} \left(\frac{U_e}{U_B} \right)^{1/5} \left(\frac{B_c}{10^4 G} \right)^{4/5} \left(\frac{\lambda_s}{\mu m} \right)^{3/5} \\ & \times \left(\frac{R_c}{10^{14} cm} \right)^{6/5} 1.4 \times 10^{14} cm \end{aligned} \quad (7)$$

R_c is the radius at which the magnetic field on the equator takes the value B_c . Taking $R_c = R_e$, B_c is the field on the equator of the photosphere. Equation (7) can then be rearranged to give

$$B_c = \left(\frac{L}{3} \right)^{-1/4} \left(\frac{U_e}{U_B} \right)^{-1/4} \left(\frac{\lambda_s}{\mu m} \right)^{-3/4} \left(\frac{R_e}{10^{14} cm} \right)^{-1/4} 6.2 \times 10^3 G \quad (8)$$

This is the field required for the plasma to be optically thick to radiation with wavelength λ_s . If $U_e \sim U_B$, the conditions for

collimation of infrared/optical/ultraviolet radiation seem to be consistent with those expected near massive black holes⁴, that is, 10^4 G at a radius of 10^{14} cm. Conditions for collimation of radio emission ($\lambda_s > 1$ cm) might also be met in active galactic nuclei (AGNs) as this would require $B_c \sim 0.1$ G at $R_c \sim 1$ pc, which is consistent with minimum-energy calculations for nuclear radio sources⁴. Pulsar fields⁵, $\sim 10^{12}$ G at $\sim 10^6$ cm, may be able to collimate radiation at even shorter wavelengths.

It is crucial that the magnetic field is well ordered. A dipole field is a natural assumption and occurs elsewhere (for example, in planetary fields) but even if the field is not dipolar, its geometry may still have the required properties. For example, if the field is generated by differential rotation of a plasma with a frozen-in field, the tangential field is likely to be enhanced near the equator where the shear is greatest, and weak on the rotation axis. A similar pattern of anisotropic absorption would then follow. The requirement of a well ordered magnetic field suggests that the model is best applied close to a compact object as the object itself or an accretion disk could provide stable anchorage for the magnetic field.

If the model is to have wide application, it has to be able to channel a large fraction of the energy generated by the central engine into beams. Energetic electrons can be produced very efficiently, as seen in some radio sources, such as Cassiopeia A (ref. 6). For the numbers quoted above, the synchrotron loss times are much shorter than other relevant timescales at infrared/optical/ultraviolet wavelengths. If the electrons lose their energy by synchrotron emission, it follows that a substantial fraction of the available energy escapes as synchrotron radiation. Although the model relies on absorption for collimation, the absorbed energy is not lost to low-energy particles, but is absorbed by energetic electrons which can re-emit the energy as synchrotron radiation. Therefore, once energy is given to energetic electrons, it is likely to remain in this form until it escapes from the plasma as synchrotron radiation. Energy transfer in the central region can take place by radiative or particle diffusion.

The model requires that the radius of the central emitting region be smaller than the maximum radius of the photosphere, but not so much smaller that the escape of radiation is negligible. The size of the emitting region might be self-regulating. If the escape of electromagnetic radiation represents the principal energy loss, then the energy stored in the central region can be expected to build up until loss becomes possible. The build-up may lead to the emitting region increasing in size, by radiation or particle diffusion for example, until its boundary intercepts the photosphere, energy escapes and the build-up is halted. Alternatively, energy could steadily be transferred to increasingly high-energy electrons until the frequency of the emitted radiation is high enough to allow it to escape. In either case, build-up proceeds until radiative loss occurs.

The model depends on the dominance of synchrotron absorption over Compton (or Thomson) scattering, which would make the radiation isotropic. The ratio of the synchrotron absorption rate, γ (equation (1)), to the Compton scattering rate (ref. 3), $\gamma_c = n_e \sigma_t c$ where n_e is the electron density and σ_t the Thomson cross-section, is

$$\frac{\gamma}{\gamma_c} = 110 \left(\frac{L}{3} \right) \left(\frac{K(\gamma_s mc^2)^{-1}}{n_e} \right) \left(\frac{B \sin \theta}{10^4 \text{ G}} \right)^{3/2} \left(\frac{\lambda_s}{\mu\text{m}} \right)^{5/2} \quad (9)$$

where γ_s is the Lorentz factor of an electron producing synchrotron emission at a characteristic wavelength λ_s , $\gamma_s = 160(B \sin \theta / 10^4 \text{ G})^{-1/2} (\lambda_s / \mu\text{m})^{-1/2}$, and $a = 2$. This implies that Compton scattering is weaker than synchrotron absorption at wavelengths around $1 \mu\text{m}$ or longer. A large number of low-energy electrons would make Compton scattering dominant, but measurements of Faraday rotation indicate that the number density of low-energy electrons is small, at least in radio-emitting regions⁷. Setting $\gamma = \gamma_c$ gives a lower limit on the wavelength of the collimated radiation, $\lambda_{\min} \sim 0.1(B/(10^4 \text{ G}))^{-3/5} \mu\text{m}$. A consequence of the requirement that Compton scattering should be weak is that X-rays should be able to escape the photosphere without scattering. Their escape is also not impeded by synchrotron absorption because absorption is weaker at short wavelengths (equation (1)).

Energetic electrons frequently have power-law energy spectra, but Ghisellini *et al.*⁸ have pointed out that the energy spectrum in an optically thick synchrotron source is more likely a relativistic maxwellian. A reasonable hypothesis is that the distribution is maxwellian with the temperature decreasing with radius. This would not qualitatively change the model. It is also likely that the electron density decreases with radius. I recalculated the shape of the photosphere for a power-law energy spectrum with density $\propto 1/r^2$ and the density at a radius $0.72R_0$ (the equatorial radius in Fig. 1) equal to that in the uniform case. The photosphere changes very little because the radial variation of absorption is dominated by its dependence on B , $\gamma \propto B^2$ with $B \propto 1/r^3$.

A well ordered magnetic field implies the possibility of strong polarization. Along the axis of the beam, by symmetry, there will be no linear polarization, only circular polarization at a level determined by the Lorentz factor of the emitting electrons. The edges of the beam will be linearly polarized because radiation emerges asymmetrically across the magnetic field and synchrotron absorption differs by a factor of ~ 7 (the ratio of the values of L in equation (1)) between the two linear polarizations. The pattern of polarization will be rotationally symmetric about the beam axis. Hence the strength of polarization and the angle of the electric vector will depend sensitively on position in the beam. In blazars¹ we may be observing compact objects along the magnetic axis and directly receiving radiation beamed from the funnel of the photosphere. This would be consistent with previous interpretations^{1,9} of blazars as AGNs viewed along the beam axis. It is observed that the intense infrared and optical radiation from blazars is strongly polarized and can vary on the timescale of a day, indicating emission from a region of dimension $\sim 10^{15}$ cm at most and implying an origin close to the compact object. Small changes in magnetic geometry could cause the observed rapid variations in polarization as polarization varies considerably within the beam. Optical emission from most AGNs other than blazars is not strongly polarized¹ which would be consistent with observation from outside the cone of the beam. The central compact object would then be obscured by the optically thick photosphere, and radiative energy from the beam would only reach us after scattering or reprocessing.

If infrared/optical/ultraviolet beams are to drive double radio sources, the beams have to interact with the plasma to produce the observed radio emission. Previous authors^{10,11} suggest that Thomson scattering might couple an electromagnetic beam to the plasma, but because Thomson scattering is probably weak

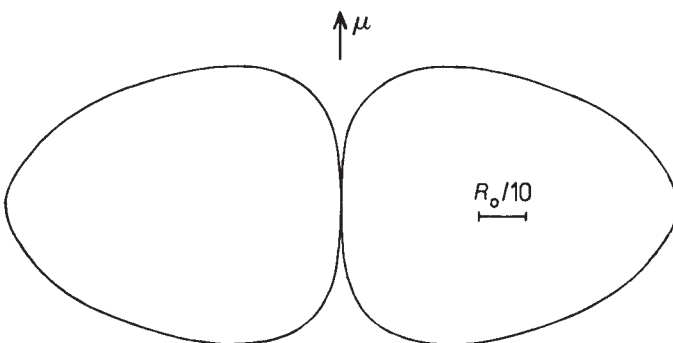


FIG. 1 Section through the photosphere. The photosphere is cylindrically symmetric about the magnetic axis, which is vertical as shown.

in the photosphere, transfer of energy and momentum from the electromagnetic beam to the plasma takes place outside the photosphere. Other possible coupling processes are synchrotron absorption itself, ponderomotive effects¹² and interaction through atomic processes, as seems likely in SS433^{13,14}. Angel and Stockman¹ note that the energy carried by electromagnetic radiation in bright blazars, if beamed, is roughly equal to the maximum luminosity of extended radio sources. This would be consistent with the supposition that energy is emitted as infrared/optical/ultraviolet radiation from the compact object, collimated by synchrotron absorption, and transferred to a plasma beam which eventually dissipates in the lobes of double radio sources. □

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Phyllosilicate veins in a CI meteorite: evidence for aqueous alteration on the parent body

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CARBONACEOUS chondrites of type CI have elemental abundances close to those of the Sun¹, and are therefore regarded as the most primitive Solar System material available for study. These meteorites consist in large part of fine-grained phyllosilicates, which account for most of the water in the meteorites (~20 wt.% H₂O). Previous studies (see, for example, ref. 2) have suggested that the phyllosilicates were formed by aqueous alteration on the meteorite parent body, but formation by direct condensation from the solar nebula has not been ruled out. Here I report the observation of fracture-filling veins of phyllosilicates in the Yamato-82162 CI chondrite. Such veins were previously known to contain only sulphates and carbonates^{3–6}. Transmission electron microscope observations show that the phyllosilicates are coherent intergrowths of iron-bearing, sodium-rich saponite and minor chlorite-like phyllosilicate; thus, they are similar to the phyllosilicates in the Orgueil CI chondrite⁷. These observations provide new evidence that the phyllosilicates in CI chondrites were probably formed by the activity of aqueous solutions on the meteorite parent body, and that phyllosilicate formation overlapped with the period of impact brecciation.

The most convincing evidence for aqueous alteration is probably the presence of veins of Mg- and Ca-sulphates and Ca-Mg carbonates in CI chondrites^{3–6}, which greatly influenced the concept of carbonaceous-chondrite evolution. The texture suggests that the sulphates and carbonates were formed elsewhere and transported to their present location in solution after or during impact brecciation and leaching events on their meteorite parent bodies. If the phyllosilicates were produced during such

a process, the meteorites would be expected to retain some evidence of this.

Yamato-82162 (Y82162) is the first CI chondrite from Antarctica to become available for study^{8–11}. Like other CI chondrites, it is a breccia composed of submillimetre to millimetre clasts. The meteorite lacks chondrules and inclusions of anhydrous silicates and consists mostly of an optically opaque matrix. The main fraction of the matrix is composed of fine-grained phyllosilicates. In addition, this meteorite contains considerable amounts of coarsely crystallized phyllosilicates. They occur as isolated clusters (50–300 µm in diameter)⁹, and more conspicuously as fracture-filling veins (Fig. 1a, b). The phyllosilicate veins vary in width from 1 to 200 µm and extend up to 500 µm. Some veins branch out to narrower veins (2–5 µm in width), forming a network. They commonly terminate at clast boundaries (Fig. 1a, b), indicating that the phyllosilicate veins were formed before some of the brecciation events that occurred on the meteorite parent body.

The coarse phyllosilicates in the veins contain large amounts of Mg, lesser amounts of Fe, and small amounts of Al and Na. No composition differences were observed between the phyllosilicates in the clusters and the veins (see Table 3 in Tomeoka *et al.*⁹), suggesting that they are probably the same and have a common origin. Our transmission electron microscope observations show that the coarse phyllosilicates occur in bundles of layers 100–1,000 Å in width. The crystals are larger than those in Orgueil⁷. They show only weak diffraction spots with broad powder rings in their selected electron diffraction patterns, however, indicating poor crystallinity.

High-resolution transmission electron microscope observations reveal that the phyllosilicates occur in coherent, disordered intergrowths of two types of layers (Fig. 2). One type has an interlayer spacing of ~10 Å, and the other has variable spacings of 13–15 Å. The latter commonly occurs as single layers and exhibits darker contrast than the former, suggesting that the dark layers contain heavier atoms than the light layers. Thus, Fe may be more concentrated in the layers of wider spacings. The 10-Å layers probably correspond to Fe-bearing saponite. Although I am less certain of the identity of the layers of wider spacings, they may be chlorite. The coherent intergrowth of the two different types of phyllosilicates is similar to that in Orgueil⁷. But in Orgueil, serpentine is the other phyllosilicate that is intergrown with saponite, and serpentine and saponite occur in approximately equal molar proportions. In contrast, the phyllosilicates in Y82162 show dominant saponite layers over chlorite-like layers, which is consistent with the results of previous microprobe analysis⁹.

Y82162 shows many mineralogical and chemical features distinct from non-Antarctic CI chondrites. There is evidence that it has experienced mild thermal metamorphism^{9,11}; the poor crystallinity of the phyllosilicates is probably ascribed to structural decomposition by heating. Y82162 lacks veins of sulphates and carbonates that are prominent in the non-Antarctic CI chondrites, and has a much higher abundance of coarse phyllosilicates (~4 vol% in modal abundance) than the non-Antarctic CI chondrites. These characteristics suggest that Y82162 has experienced a late history distinct from that of the known non-Antarctic CI chondrites.

Previous studies suggested that the aqueous alteration experienced by the CI chondrites was a multistage process and that the sulphate veins formed in a relatively late alteration stage^{3–5,7}. In Orgueil, there is evidence that the coarse phyllosilicates were probably altered to matrix during the aqueous alteration that produced the sulphate veins⁷. From these observations and evidence, Tomeoka *et al.*⁹ suggested that Y82162 had not undergone the late aqueous alteration stage, so retaining abundant coarse phyllosilicates. The new results show that the coarse phyllosilicates in the CI chondrites were probably initially produced as vein fillings during aqueous alteration. Therefore, it is plausible that the fine phyllosilicates in the matrices of CI

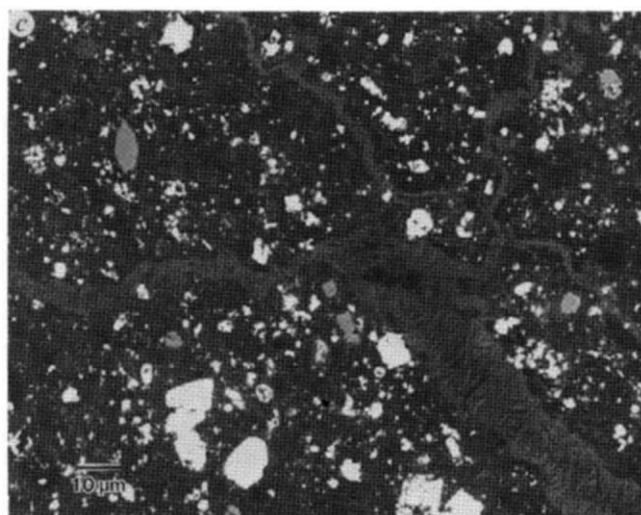
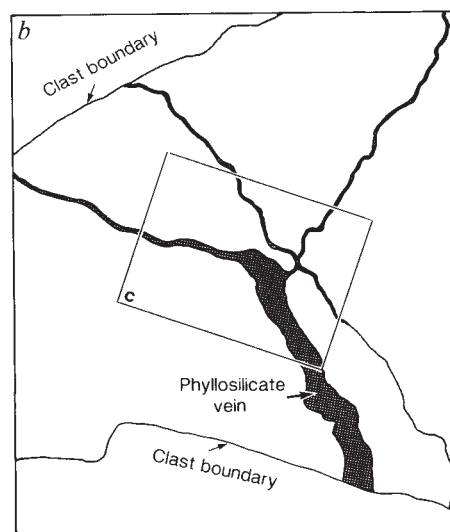
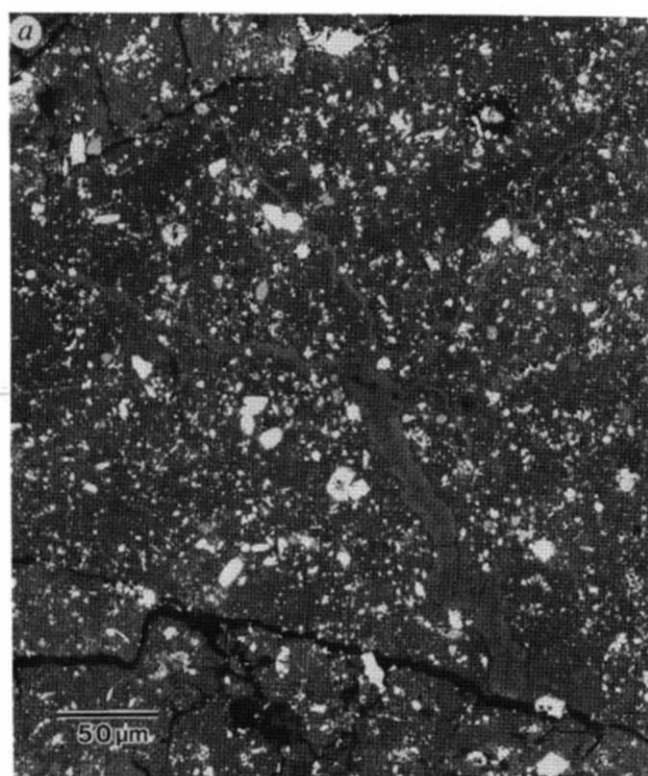


FIG. 1 *a*, A portion of a clast containing phyllosilicate veins (back-scattered scanning electron microscope image). Phyllosilicate veins terminate at clast boundaries. Small pyrrhotite grains (bright) are dispersed in the matrix. *b*, Illustration showing phyllosilicate veins and clast boundaries. *c*, A high-magnification image of the central portion in *a*, which is boxed by solid lines in *b*.

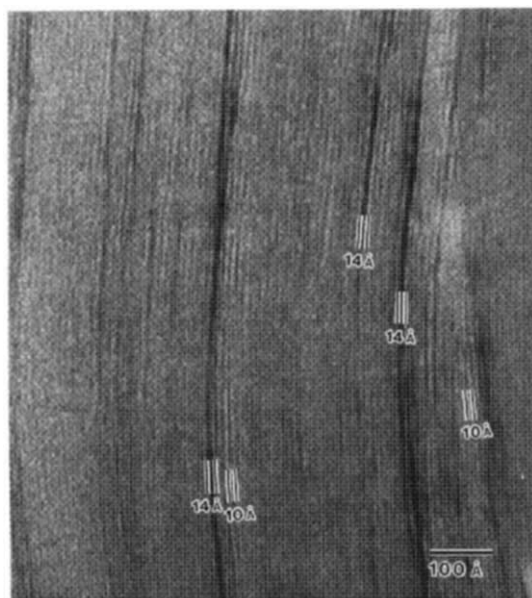


FIG. 2 A high-resolution transmission electron microscope image of the phyllosilicates in Yamato-82162. Saponite (10-Å layers) and chlorite-like phyllosilicate (14-Å layers) are coherently intergrown.

chondrites developed during repetitive vein formation and degradation by the activity of aqueous solutions. Compositions and Eh (oxidation potential) pH conditions of the aqueous solutions probably differed considerably during different stages of alteration, thus resulting in a variety of vein mineralizations.

The presence of phyllosilicate veins has another important implication. Despite the indication of aqueous alteration in the meteorite parent body, it was uncertain whether the bulk of matrix alteration occurred in asteroidal regoliths or interiors¹². The clastic texture of CI chondrites such as that shown in Fig. 1 was probably produced by impact-induced regolith turnover and brecciation. It is evident from the texture that the fracture space now being filled by the phyllosilicates was formed during the regolith process and that brecciation occurred after the phyllosilicate-vein formation. These observations suggest that the aqueous alteration to produce phyllosilicates occurred in regoliths, and was contemporaneous with the period of regolith gardening. Because the water probably accreted onto the parent body as ice¹³, a heating event is required for melting. If our hypothesis that alteration was contemporaneous with regolith gardening is valid, it is conceivable that the heat was supplied *in situ* by shock impact on the regolith rather than by decay of radionuclides from internal regions of the parent body. □

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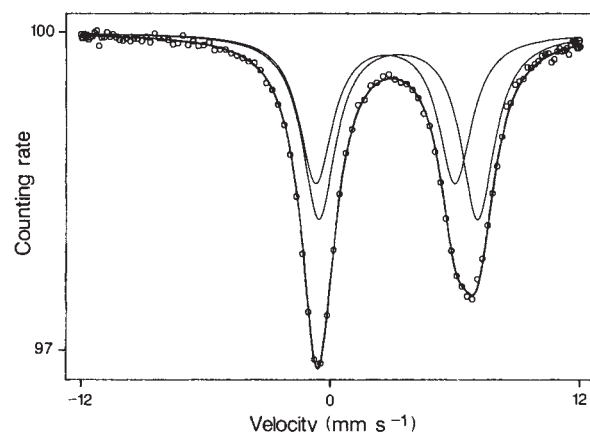


FIG. 1 ^{197}Au Mössbauer spectrum of crystalline $\{[(\text{C}_6\text{H}_5)_3\text{PAu}]_5\text{N}\}^{2+}(\text{BF}_4^-)_2 \cdot (\text{C}_4\text{H}_8\text{O})_2$ at 4 K.

Electron-deficient bonding at pentacoordinate nitrogen

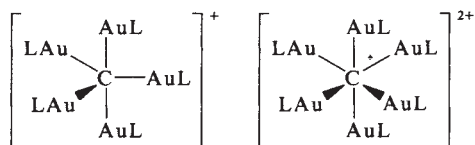
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IT has been demonstrated recently that pentacoordinate and hexacoordinate carbon atoms can be prepared in complexes containing two-coordinate gold(I) species^{1–3}. This unusual environment for carbon can be ascribed to the tendency of gold(I) to form clusters with short intramolecular contacts between the metal atoms^{4,5}; the effect, termed 'aurophilicity', suggests the possibility of forming polyaurated species with the metal atoms clustering about a central element. Here we report that nitrogen, as well as carbon, can demonstrate unusually high coordination when included as the central atom in such complexes. We have prepared an aurated dication in which the nitrogen exhibits fivefold coordination. In keeping with theoretical predictions, we anticipate that it may prove possible to synthesize similar compounds containing other central elements, such as boron.

Gold has a unique position in the periodic table, as evident from such characteristic properties as high electrochemical potential, high electron affinity (electronegativity), small atomic radius, and low coordination number in chemical compounds^{6,7}. Much attention has been given recently to the tendency of two-coordinate gold atoms in Au(I) complexes to form dimers, chains or layers^{4,5}; similar but intramolecular gold–gold bonding has been observed in di- or polynuclear complexes^{8–10}. This 'aurophilic' effect is the result of secondary bonding between closed-shell d^{10} centres (Au^+).

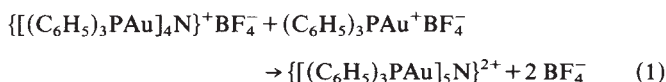
Studies of aurophilicity in polyaurated species have led to the discovery of novel polyauromethane cations containing penta- and even hexacoordinate carbon atoms. Examples are the homoleptic species $\{[(\text{C}_6\text{H}_5)_3\text{PAu}]_5\text{C}\}^+$ and $\{[(\text{C}_6\text{H}_5)_3\text{PAu}]_6\text{C}\}^{2+}$ (see structures below) and also penta-coordinate carbon cations of the type $\{[\text{R}_3\text{PAu}]_4\text{CR}\}^+$ (ref. 3).



These results not only confirmed earlier theoretical predictions¹¹, but also helped to explain certain inconsistencies in the literature^{12–14}. Recent theoretical work indicates that a quantum-chemical treatment of the electronic structures of these compounds is possible^{15,16}.

We now report the preparation and structure of a novel penta-aurated nitrogen dication, derived from a classical tetra-

kis(phosphinegold)ammonium monocation^{17,18}, $\{[\text{LAu}]_4\text{N}\}^+$. Tetrakis(triphenylphosphinegold)ammonium tetrafluoroborate was found to add one equivalent of (triphenylphosphine)gold tetrafluoroborate in a hexamethylphosphoric triamide/tetrahydrofuran mixed solvent at room temperature. We obtained high yields of a colourless crystalline material which was identified as pentakis(triphenylphosphinegold)ammonium(2+) bis(tetrafluoroborate) (Chemical Abstracts nomenclature: μ^5 -nitrido-pentakis(triphenylphosphine)-pentagold(I)-(2+)):



Samples recrystallized from $\text{CH}_2\text{Cl}_2/\text{THF}$ contain two equivalents of crystalline tetrahydrofuran, which can be removed by drying *in vacuo*. The composition of the product was confirmed by element analysis and mass spectrometry. Field-desorption mass spectrometry from dichloromethane solution showed a parent peak at $m/z = 1,155.2$ (M^{2+} , 100%), accompanied only by a peak at $m/z = 722.4$ from $[(\text{C}_6\text{H}_5)_3\text{P}]_2\text{AuH}^+$ (2.5%). The ionic character of the compound is also implied by its high solubility in polar solvents.

Infrared spectra from samples on KBr confirm the presence of triphenylphosphine and tetrafluoroborate units by comparison of 'fingerprint bands' documented for related systems. ^1H , ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectra (dichloromethane solutions at 20 °C) show that the triphenylphosphine groups are equivalent (possibly through time averaging). The ^{31}P chemical shift ($\delta = 25.3$ p.p.m. relative to external 85% aqueous H_3PO_4) is close to the values found for the aurated methane cations, but different from that of the starting materials. The fine-structure of the ^{13}C resonances suggests high-order CPAuNAuPC coupling¹, but is not fully resolved. The NMR experiments consistently show the compound to be diamagnetic.

The ^{197}Au Mössbauer spectrum (Fig. 1) (crystalline powder at 4 K) gives an overlapping set of two doublets, with isomer shifts and quadrupole coupling constants that suggest the presence of Au(I) centres in two different environments. Interestingly, the ratio of intensities of the quadrupole doublets is close to 3:2; deviation from this value is easily explained by small differences in the recoil fractions¹⁹.

Together with the analytical and spectroscopic data, this result suggests an ionic structure consisting of a nitrogen-centred dication surrounded by a trigonal bipyramid of five gold atoms. A single-crystal X-ray structure analysis confirms this structure (crystallographic details in box). The monoclinic cell ($a = 15.242(2)$, $b = 23.638(3)$, $c = 26.518(3)$ Å, $\beta = 94.83(1)^\circ$, $Z = 4$)

Crystal structure data

$C_{90}H_{75}Au_5B_2F_8NP_5 \cdot 2C_4H_8O$, $M_r = 2628.17$, monoclinic, space group $P2_1/n$ (No. 14), $a = 15.242(2)$, $b = 23.638(3)$, $c = 26.518(3)$ Å, $\beta = 94.83(1)^\circ$, $V = 9520.3$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.833$ g cm⁻³, $\mu(\text{Mo K}\alpha) = 78.0$ cm⁻¹, $F(000) = 5016$, $T = 23$ °C. 16,632 unique reflections to $(\sin \theta/\lambda)_{\text{max}} = 0.60$ Å⁻¹, 9,358 'observed' with $F_o \geq 4.0\sigma(F_o)$. Empirical absorption correction (relative transmission: 0.58–1.00). Solution by direct methods (SHELXS-86); $R(R_w) = 0.041(0.027)$, $w = 1/\sigma^2(F_o)$, blocked-matrix least-squares refinement (SHELX-76). 1,090 refined parameters, anisotropic, H constant, $\Delta\rho_{\text{fin}}$ (max/min) $+0.90$ -0.90 e Å⁻³. Further details of the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, D-7514 Eggenstein-Leopoldshafen 2 (FRG), on quoting the depository number CSD-54550, the names of the authors, and the journal citation.

contains one dication $\{[(C_6H_5)_3PAu]_5N\}^{2+}$, two anions BF_4^- , and two tetrahydrofuran molecules C_4H_8O in the asymmetric unit. The structure was solved using standard computational methods, with the refinement (based on 16,632 independent reflections and 1,090 parameters) converging at a goodness-of-fit parameter $R(R_w) = 0.041(0.027)$. The molecular structure of the dication (with no crystallographic symmetry) is shown in Fig. 2. It contains a pentacoordinate nitrogen atom surrounded by five gold atoms in a distorted trigonal bipyramid. The gold–nitrogen distances are shorter for the equatorial metal atoms (N–Au3 = 2.051(7), N–Au4 = 2.081(6), N–Au5 = 2.066(7) Å) than for the axial metal atoms (N–Au1 = 2.116(6), N–Au2 = 2.112(6) Å). The Au1–N–Au2 angle of $174.9(4)^\circ$ is close to the ideal 180° for a regular trigonal bipyramid, but the equatorial angles deviate more strongly from 120° : Au3–N–Au4 =

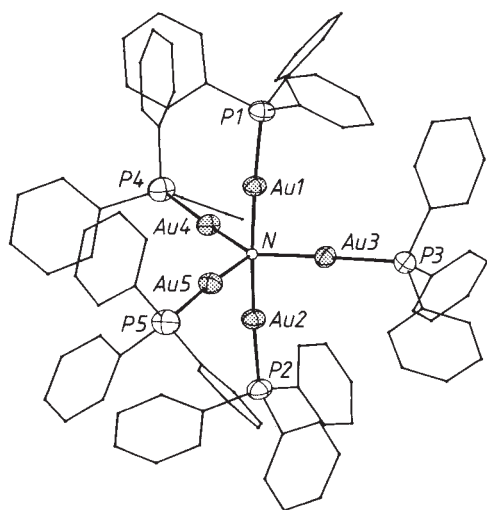


FIG. 2 Structure of the dication $\{[(C_6H_5)_3PAu]_5N\}^{2+}$ in crystals of its bis(tetrafluoroborate) bis(tetrahydrofuran) salt, as determined by X-ray diffraction. Except for the gold and phosphorus atoms (50% probability ellipsoids) the atoms have been drawn with arbitrary radii. Hydrogen atoms have been omitted for clarity. Selected distances (Å) and angles (see also text): Au1–Au3 = 2.933(1), Au1–Au4 = 2.910(1), Au1–Au5 = 3.074(1), Au2–Au3 = 2.885(1), Au2–Au4 = 2.984(1), Au2–Au5 = 2.965(1); Au1–P1 = 2.248(3), Au2–P2 = 2.244(3), Au3–P3 = 2.244(3), Au4–P4 = 2.248(3), Au5–P5 = 2.242(3); P1–Au1–N = $174.3(2)^\circ$, P2–Au2–N = $176.8(2)^\circ$, P3–Au3–N = $177.8(2)^\circ$, P4–Au4–N = $176.5(2)^\circ$, P5–Au5–N = $174.6(2)^\circ$.

$129.0(3)^\circ$, Au4–N–Au5 = $109.6(3)^\circ$, Au3–N–Au5 = $121.4(3)^\circ$. The sum of these angles is 360.0° , indicating that the nitrogen atom and the triplet Au3, Au4, Au5 are coplanar. These distortions are possibly owing to steric crowding of the 15 phenyl groups. The gold atoms are all two-coordinate, with a phosphorus atom as the second ligand. The N–Au–P angles are all close to 180° , as expected for twofold coordination of a d^{10} metal centre. Neither the counterions nor the solvent molecules show structural irregularities or unusual intermolecular (interionic) contacts.

Reaction (1) involves electrophilic attack at the nitrogen atom of an ammonium cation, which is without precedent. The $(C_6H_5)_3PAu^+$ electrophile does not induce substitution at the nitrogen centre of the $\{[(C_6H_5)_3PAu]_4N\}^+$ cation through a pentacoordinate or other transition state, but is accommodated as a fifth ligand to give an extremely stable species. Although a qualitative description of the unusual bonding in the NAu_5 core is possible by using a simplified molecular orbital diagram for an electron-deficient species of D_{3h} symmetry, as already suggested as a rationalization of the $(LAu)_5C^+$ analogue², it seems to be necessary to include peripheral metal–metal interactions involving the d^{10} closed shell. Such interactions have previously been incorporated in the description of other short contacts between coinage metals^{20–22} with particular emphasis on relativistic effects^{15,16}. In models that take into account the novel features of structure and bonding of polyaurated species, aggregation of gold atoms at small main-group-element centres (aurophilicity) is predicted to be a very general phenomenon, which our synthesis of the nitrogen dication seems to confirm.

To put this result into perspective, we draw attention to a few related, hypothetical nitrogen-centred species. Given the 'isobal' relationship^{23,24} (that is, the similarity in bonding) between LAu^+ and H^+ or Li^+ , formation of the cations NH_5^{2+} and NLi_5^{2+} should be feasible in principle. To the best of our knowledge, however, none of these has been investigated theoretically, and protonation of the ammonium cation has not been reported. Whereas experiments^{25–29} and calculations^{30–33} are well known for the related cases of CH_5^+ and CLi_n^q ($n = 5$ or 6, $q = \pm 1$ or ± 2), information on the nitrogen analogues is still very limited^{34,35}. Preparation of the trication $\{[(C_6H_5)_3PAu]_6N\}^{3+}$, via a different route, has been reported recently (A. Brodbeck and J. Strähle, personal communication), and the special role of Au(I) in the formation of element-centred clusters now seems to be well established for carbon and nitrogen. Work on analogous boron compounds is in progress. □

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Bistability of CCN concentrations and thermodynamics in the cloud-topped boundary layer

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RECENT discussions of the effect of cloud condensation nuclei (CCN) in controlling the albedo of marine stratus cloud and hence global climate^{1–3} have raised questions concerning the factors that control CCN populations and how (or whether) such factors should be included in climate models⁴. The observed near-constancy⁵ of CCN concentrations in remote marine air is remarkable in view of the wide variety of possible conditions, the range over which they might be expected to vary, and the considerable difference between marine and continental CCN concentrations. Here we explain this relative lack of variability, thought to be due to a balance of source and sink processes^{6,7}, in a simple model of the marine cloud-topped boundary layer. Solutions to this model give two stable CCN concentration regimes—one corresponding to the low concentration observed over the ocean, the other corresponding to the higher concentration observed over land—each dominated by a different sink mechanism. The two regimes have very different optical properties, and thus may be of climatic significance.

We consider here the horizontally uniform, marine cloud-topped boundary layer (CTBL), a well mixed layer of depth ~ 1 km between the ocean surface and a sharp temperature inversion, which is characteristic of the lower atmosphere over much of the subtropical oceans. Cloud fills the upper 200–300 m of this layer, which we characterize by three parameters, θ_M^* , Q_M^* and N_M . The conserved parameter θ_M^* , which is related to the mean temperature in the layer, is observed to be approximately well mixed (or constant with height) in the CTBL (thus the term mixed layer). Q_M^* is a measure of the total water (vapour + liquid) and, like θ_M^* , is conserved during phase change. Although these parameters are conserved during phase changes and mixing within the boundary layer, they can be altered by surface fluxes and large-scale subsidence (or sinking motion), for example. The subscript M denotes a mixed-layer value and, strictly speaking, defines an average over the depth of the CTBL (that is, an average from the surface to the inversion that caps the boundary layer and the cloud deck). The asterisks denote saturation point values. N_M is the layer-averaged total CCN particle number concentration.

We prescribe the layer depth, z_i , the properties of the surface and the upper air (assumed horizontally uniform), the CCN source strength, and the effective vertical-transport velocities responsible for the fluxes at the upper and lower interfaces of the CTBL. We seek the steady-state solutions of the system of coupled conservation equations for these three layer parameters.

Gas-to-particle conversion, for example $\text{SO}_2 \rightarrow \text{SO}_4$, provides small particles which grow to CCN size ($\geq 0.05 \mu\text{m}$) yielding a new CCN production rate of $S_0(\text{m}^3\text{s})^{-1}$ in the lower troposphere. Here S_0 is the production rate of water-soluble particles that are large enough, $0.05 \leq r \leq 1 \mu\text{m}$ (r , radius), to nucleate cloud droplets at typical supersaturations in clouds ($\sim 0.1\%$). (It is important to distinguish this rate from the rate of production of much smaller particles directly by gas-to-particle conversion; although the latter is as yet very poorly known, we shall use circumstantial evidence to estimate the former.) The CCN subsequently undergo processes that modify their sizes, and those processes leading to their eventual removal from the atmosphere may be size-dependent. Here, however, we ignore these size dependences and derive a crude conservation equation for N_M .

In the cloudless atmosphere the major sink for N_M is Brownian diffusion, which causes their coagulation at a rate βN_M^2 . In the presence of clouds, the conservation equation for N_M becomes more complicated. For our crude model we can derive an approximate equation in which the supersaturation is high enough for all CCN to be activated. This simplification is justified by observations in clean marine air⁸. (At higher values of N_M , the source rate and/or fraction of particles activated may be dependent on N_M , complicating this simple picture. Here we neglect this complication and focus primarily on the properties of the CTBL at lower values of N_M .) In this case, the chief particle removal processes are coagulation of dry particles, coalescence of cloud drops, coalescence of cloud and drizzle drops, collection of particles below cloud by falling drizzle drops, removal of drizzle when it hits the surface and vertical transport out of the layer. As the variation in β is small (it varies by about a factor of two) over the range $0.1 \leq r \leq 10 \mu\text{m}$ (ref. 9), we consider it constant over this range. Thus, assuming the CCN concentration does not vary much over the layer

$$\frac{dN_M}{dt} = S_0 - \beta N_M^2 - \dot{N}_{\text{collisions}} - \dot{N}_{\text{surface}} - \dot{N}_{\text{transport}} \quad (1)$$

Here we use $\beta = 10^{-15} \text{ m}^3 \text{ s}^{-1}$. $\dot{N}_{\text{collisions}}$ represents the sink due to collisions of cloud droplets with drizzle drops. At any height z , $\dot{N}_{\text{collisions}}(z) = \frac{1}{2} N_M \pi r_d^2 E(r_d, r) f(z) / m_d$ where r_d is the rain-drop radius, $E(r_d, r)$ the collection efficiency for collisions of raindrops and small particles of radius r , $f(z)$ is the precipitation flux of liquid water at height z in $\text{kg H}_2\text{O m}^{-2} \text{ s}^{-1}$, and m_d the average drizzle drop mass. The turbulent fluxes at the surfaces are given in terms of an effective velocity w_0 and the subsidence velocity is w_T . Thus

$$\begin{aligned} \frac{dN_M}{dt} = S_0 - \beta N_M^2 - \frac{\frac{1}{2} N_M \pi r_d^2 \int E(r_d, \bar{r}_c) f(z) dz + f(0)}{m_d z_i} \\ + \frac{w_0(N_0 - N_M) + w_T(N_T - N_M)}{z_i} \end{aligned} \quad (2)$$

where $\bar{r}_c$ is the mean droplet radius at cloudtop, N_0 and N_T are the number concentrations at the surface and upper boundaries of the CTBL, respectively and the integral goes over the entire CTBL.

Lacking suitable data, we use the parameterization by Turton and Nicholls¹⁰ of their North Sea data, in which the drizzle flux $f(z)$ is a constant, f_0 , in cloud and decreases linearly with pressure below cloud base. The flux f_0 increases with cloud thickness and average liquid-water content $\bar{w}_l$. It rises with $\bar{r}_c$ ($\sim (\bar{w}_l / N_M)^{1/3}$) for $\bar{r}_c < 10 \mu\text{m}$ and is independent of $\bar{r}_c$ for larger sizes. We complete the parameterization by setting $E(r_d, \bar{r}_c) = 1$ for $\bar{r}_c > 10 \mu\text{m}$, $E(r_d, \bar{r}_c)$ diminishing as $\bar{r}_c^3$ for smaller droplets. We choose radii r_{max} , r_{min} such that $r_d = r_{\text{max}}$ if $\bar{r}_c < r_{\text{max}}$, $r_d = \bar{r}_c$ if $\bar{r}_c > r_{\text{max}}$, and $f(z) = 0$, if $\bar{r}_c < r_{\text{min}}$. Variation of r_{min} and r_{max} within realistic ranges had little effect on the qualitative features of the results.

We use equation (2) with these parameterizations and approximations in conjunction with the conservation equations

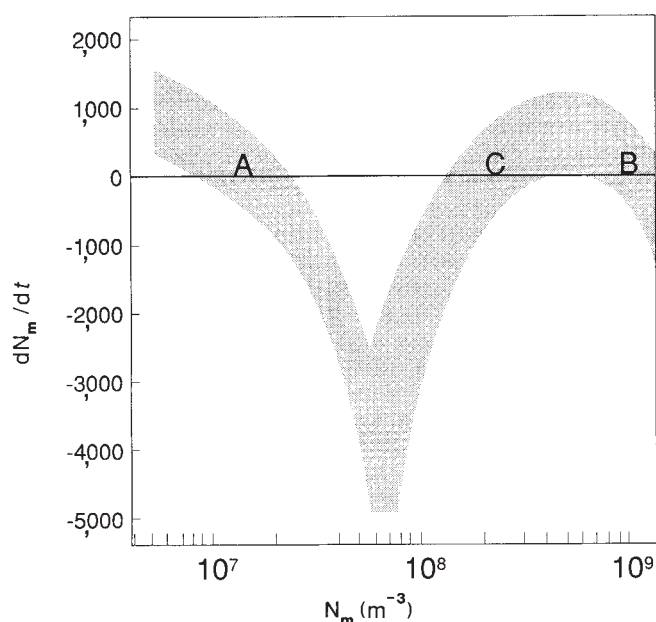


FIG. 1 Variation of N_M according to equation (1), with fixed boundary conditions. The envelope comprises variations in S_0 from 800 to 2,000 ($\text{m}^3 \text{s}^{-1}$), in boundary-layer thickness from 800 to 1,000 m, and in cloud thickness from 250 to 300 m. See text for definition of points A, B and C.

for Q_M^* and θ_M^* ¹¹⁻¹³ to investigate the steady-state solutions of the CTBL. For ease of presentation, we put the density of air equal to unity, and thus write

$$\frac{dQ_M^*}{dt} = \frac{w_0(Q_0^* - Q_M^*) + w_T(Q_T^* - Q_M^*) - f(0)}{z_i} \quad (3)$$

Here Q_0^* and Q_T^* are the total water contents at the surface and in the upper-level air.

The equation for θ_M^* is

$$\frac{d\theta_M^*}{dt} = \frac{w_0(\theta_0^* - \theta_M^*) + w_T(\theta_T^* - \theta_M^*) - (F - Lf(0))/c_p}{z_i} \quad (4)$$

where L (J kg^{-1}) is the latent heat of evaporation, F (W m^{-2}) the net outgoing (long-wave and short-wave) radiative flux and c_p is the constant-pressure heat capacity of moist air. F is a function of N_M and $\bar{w}_l$ but in this first examination of the precipitating system we neglect this dependence. We calculate the cloudbase height from Q_M^* and θ_M^* ; the liquid-water content is a linear function of pressure above the base of the cloud.

It is important to note the weaknesses of the system of equations (2)–(4) as a representation of the marine boundary layer. It assumes a well-mixed layer no matter what the precipitation rate, although precipitation can decouple the cloud and sub-cloud layers because it induces latent heating in the cloud and evaporative cooling below¹⁰. This may lead to cloud break-up and a situation of fractional cloudiness which our simple model cannot describe. In addition, it assumes all particles larger than one critical size are activated, that all cloud drops have the same size, and it uses a very crude parameterization of the precipitation—one devised for the North Sea, and not the subtropical oceans to which we now apply our model. Moreover, as stated above, we do not consider the dependence of F on the other parameters. Despite these deficiencies in the model, it offers a first approximation to this complex system and we feel that its steady-state behaviour is a guide to behaviour in the real subtropical marine atmosphere.

We can crudely estimate the magnitude of S_0 by noting that the residence time for the precursor gas, SO_2 is $\sim 1 \times 10^5 \text{ s}$, and

that a typical concentration in the boundary layer is $\sim 0.5 \text{ nmol m}^{-3}$ (ref. 14). The average reaction rate is therefore $\sim 5 \times 10^{-15} \text{ mol m}^{-3} \text{ s}^{-1}$. The marine CCN number concentration as inferred by electron microscope¹⁵ is dominated by sulphate particles of $\sim 50 \text{ nm}$ in radius, or $\sim 5 \times 10^{-18} \text{ mol SO}_4$ per particle, assuming a particle density of 1 g cm^{-3} . Thus, the oxidation of SO_2 can support a new CCN formation rate of $\sim 5 \times 10^{-15} / 5 \times 10^{-18} = 1,000 \text{ particles m}^{-3} \text{ s}^{-1}$, assuming that oxidation followed by nucleation scavenging represents the main removal pathway for SO_2 . We use this value as a starting point for tests of the sensitivity of our steady-state solutions to variations in source strengths and other parameters of the system. This production rate of CCN from chemical reactions probably exceeds that from wind-blown sea salt by a factor of 10–100 (ref. 2).

Figure 1 shows dN_M/dt plotted against N_M calculated from equation (2) for a range of cloud thicknesses and source strengths assuming constant thermodynamic parameters and that $N_0 = N_T = N_M$. For the lowest S_0 shown, the only stable solution is at low N_M (here, $\leq 10^6$ – 10^7 m^{-3}), where dry particle coagulation is inoperative and the source, S_0 , is primarily balanced by the coalescence of cloud drops with drizzle drops (point A). Cases with $800 \leq S_0 \leq 2,000 \text{ m}^3 \text{ s}^{-1}$ also have the low N_M solution. It is interesting that these stable solutions have CCN concentrations closely resembling those in the remote marine CTBL. For higher S_0 , there is a second stable solution (point B) at high N_M ($\sim 10^9 \text{ m}^{-3}$), where the precipitation sink is not operating and the source is balanced by coagulation and coalescence. For even higher source strengths (not shown) this is the only stable solution; precipitation cannot balance the production rate at low N_M . Note that the third intermediate solution $dN_M/dt = 0$ for high S_0 (point C) corresponds to an unstable equilibrium and has no particular physical significance. The depth and breadth of the minimum increases with cloud depth, illustrating the need to consider the coupled system in order to find a physically self-consistent picture of the CTBL; increasing cloud thickness increases the precipitation sink relative to the other terms and results in a widening of the gap between the steady-state regimes. The cusp in the curve of Fig. 1 occurs at $\bar{r}_c = 10 \mu\text{m}$, where E and f_0 reach their maximum values. Below-cloud scavenging widens and deepens the negative portions of the curve. The sharp minimum is an artefact of our particular parameterization but the overall features of Fig. 1, namely, the existence of two possible steady-state regimes separated by a gap, or ‘drizzlepause’, are independent of parameterization.

Our procedure for examining the solutions of the system of equations (2)–(4) is to use the solutions of equation (2) (with fixed Q_M^* , θ_M^*) to estimate the locations of the stable regimes. We then vary the initial conditions for the three dependent variables around these locations, and integrate the equations forward using simple forward differencing until prescribed convergence limits are met. These integrations do not represent time development of the layer, because we maintain constant boundary conditions, including boundary-layer depth. They are rather a means to mathematically reach the steady states of the system under those imposed boundary conditions. Figure 2a and b show the results of this procedure. In all runs $w_0 = 0.01 \text{ m s}^{-1}$ and $F = 30 \text{ W m}^{-2}$. This yields one, or at most, two steady states in the range of parameters typical of the marine subtropical boundary layer. Figure 2a shows the variation with S_0 of the low- N_M steady-state regime, in which precipitation is the dominant sink. This regime appears at low particle source strengths, high sea surface temperatures and/or deep boundary layers. Typical values of the precipitation rate at the surface in this regime are 10^{-6} – $10^{-5} \text{ kg m}^{-2} \text{ s}^{-1}$, or ~ 30 – 400 mm yr^{-1} . The high- N_M regime, in which the precipitation sink is relatively less important, appears at high particle source strengths, shallow boundary layers and/or low sea surface temperatures. Runs not shown here reveal that the high- N_M regime appears at lower values of S_0 for warmer air aloft and/or for high subsidence

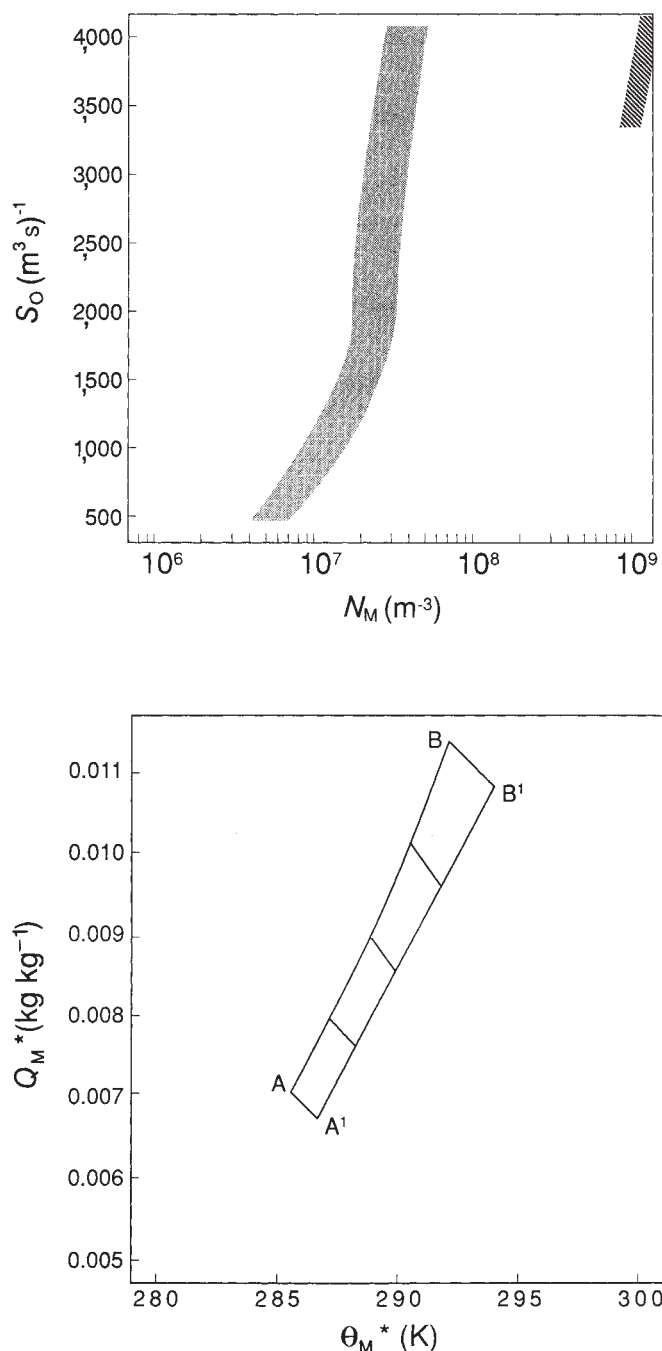


FIG. 2 a, Variation of steady-state values of N_M with source strength, boundary-layer thickness and surface temperature. All points shown correspond to $w_0 = 0.01 \text{ m s}^{-1}$, $w_T = 0.003 \text{ m s}^{-1}$, $\theta_T = 298 \text{ K}$, $Q_T = 0.001$, $F = 30 \text{ W m}^{-2}$. S_0 varied from 500 to $4,000 \text{ (m}^3 \text{s}^{-1})$. The wider, diagonally hatched envelope corresponds to $z_i = 1,000$ m and 800 m and variation of θ_0 from 288 K to 292 K. Shallower and/or cooler layers correspond to higher values of N_M (for given S_0) than deeper, warmer layers. The thinner, vertically hatched region corresponds to steady states that appear at high S_0 , low surface temperature and the lower value of z_i . b, Variation of steady-state values of θ_M^* , Q_M^* for the same range of source strengths, surface temperatures and layer thicknesses as in (a) in the low- N_M steady state. The variation A–B corresponds to variation of θ_0 from 285 to 293 K, whereas the variations indicated by the lines A–A', B–B', for example, represent variations in layer thickness from 1,000 to 800 m and variations in S_0 from 500 to $2,000 \text{ (m}^3 \text{s}^{-1})$.

rates; that is, for meteorological conditions in which precipitation is inhibited. For a range of realistic conditions both the low- and the high- N_M steady states are possible.

Figure 2b shows the variation of the thermodynamic parameters of the CTBL with changes in sea surface temperature, cloud-top height and CCN-source rate, S_0 , throughout a range in which only the low- N_M precipitation-dominated regime is present. This shows an approximately linear dependence of both Q_M^* and θ_M^* on the sea surface temperature; they are less sensitive to, but not independent of, z_i and S_0 .

Note that the subsidence velocity w_T is often diagnosed from observations of cloud-base height, layer thickness and boundary conditions, through equations (3) and (4). Our results show that neglect of precipitation will lead to an overestimation of w_T in such analyses.

We have shown that in a simple model of the uniform, well mixed boundary layer there are two, quite different stable regimes for the Q_M^* , θ_M^* , N_M system, suggesting that both the relatively small values and the constancy of CCN number concentration, N_M over the marine atmosphere are due to the balance between the production (S_0) and removal by precipitation.

We have made many simplifications in this model, such as neglect of vertical transport and the radiation feedbacks, which must be added for a comprehensive study of the steady-state regimes of the well mixed CTBL. Liou and Ou¹⁶ have used a one-dimensional model with externally prescribed CCN concentrations to illustrate the importance of particle number in regulating global climate, as suggested by Albrecht^{3,4}. Our results suggest further that the CCN number concentration itself is determined by both chemical and climate parameters and cannot be prescribed independently.

Finally, it is interesting to note that, *a priori*, the existence of two stable regimes in the marine stratus clouds of the globe could also correspond to two different climate regimes. At present the marine stratiform clouds seem to be in a low- N_M state, and their albedo is relatively low (0.3–0.7). A shift to the high- N_M state could cause a substantial decrease in the solar energy absorbed by the system because the global heat balance is sensitive to the albedo of these clouds and because they cover ~25% of the Earth's surface¹⁷. The calculations discussed here suggest that this shift may come about through elevated source strengths or if atmospheric conditions suppress precipitation over time periods sufficiently long for a new equilibrium to be established beyond the 'drizzlepause'. Our studies do not provide information on the timescales for this adjustment to, or transition between, steady-state regimes, as we have not allowed the layer thickness or the boundary conditions to vary with time. However, the conditions favouring the high- N_M regime are likely to be prevalent in situations in which cloud cover is low. The next modelling step is to extend these budget studies to the case of a non-well-mixed boundary layer, in which the water content has a role in determining the fraction of sky covered by cloud. Although the CCN concentrations in the high- N_M regime resemble those in present continental atmospheres, our model, as it stands, cannot realistically represent this case, and its further extension in that direction would be desirable. We also require simultaneous observational studies of the microphysical structure, albedo and CTBL structure, together with both background and anthropogenically enhanced CCN concentrations, to test the present model and to explore the possibility for observation of a transition to the high- N_M state. □

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Seasonal rainfall in the Sinai Desert during the late Quaternary inferred from fluorescent bands in fossil corals

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SKELETAL bands of alternating high and low density in massive coral species have been used to record their growth history^{1,2}. In the Red Sea, living colonies of the genus *Porites* deposit low-density skeletal bands during summer and high-density bands during winter³. Additionally, yellow-green fluorescence can sometimes be seen in these massive corals, imparted to them by the incorporation of humic material carried by coastal runoff⁴. Annual banding of fluorescent sequences in living scleractinian corals has proved to be useful in the study of terrestrial runoff in the near-shore environment^{5,6}. Here we report the finding of similar yellow-green fluorescent bands in fossil *Porites* from late Quaternary reef terraces in southern Sinai, which are absent from living *Porites* in the nearby fringing reefs. The periodic sequences of the fluorescing humics were found to be superimposed on the low-density sub-bands of the fossil corals. We interpret our observations as evidence that, during the late Quaternary reef-forming peaks, the climate was wetter than the extreme desert conditions now prevailing, with a possible summer rainfall regime.

The coastline along the Gulf of Eilat and the southern Sinai Peninsula is fringed by a narrow belt of modern coral reefs^{7,8} (Fig. 1a,b). A well preserved belt of three elevated fossil-reef terraces, up to 35 m above mean sea level, stretches along the coast of southern Sinai⁹ (Fig. 1c). Samples were dated as follows: the Upper Terrace (labelled I in Fig. 1c) as older than 250 kyr (1 kyr = 1000 years); the Middle Terrace (II) as ~140–200 kyr the Lower Terrace (III) as 108–140 kyr; and the modern offshore fringing reef (IV) as 10,000 years and younger^{10–12}. These terraces were formed during periods of late Quaternary high-stand sea levels (G.G., J. Kronfeld and B. Buchbinder, manuscript in preparation).

Of 26 samples of the fossil *Porites* collected from the three elevated coral terraces, 22 samples showed degrees of fluorescent banding when sections were irradiated with long-wave (~360 nm) ultraviolet light. A comparable number of living *Porites* from the modern reef did not have distinct fluorescent banding.

High-performance liquid chromatography analysis¹³ (Table 1) indicated that the source of fluorescence was humic acid that

had been incorporated into the coral skeleton, as already found in in-shore corals from the Great Barrier Reef of Australia⁴. Fluorometric analysis of the fossil corals showed that the emitted light spectrum of a solid coral skeletal sample had the broad-band shape characteristic of organic luminescence, rather than the sharp bands associated with purely inorganic luminescence. These results indicate that the luminescence of fossil corals is governed by the humic acid in the coral skeletal matter.

Fossil corals exhibiting the best fluorescent banding sequences were selected for detailed analysis and compared with a randomly selected coral from the modern reef (Table 1). Quantitative analysis of humic acid by HPLC (Table 1) showed that the fluorescent bands of the fossil coral samples (labelled Fos4 and 1389) were richer in humic acid than the adjacent non-fluorescent bands. This contrasted with a living coral (labelled S21)

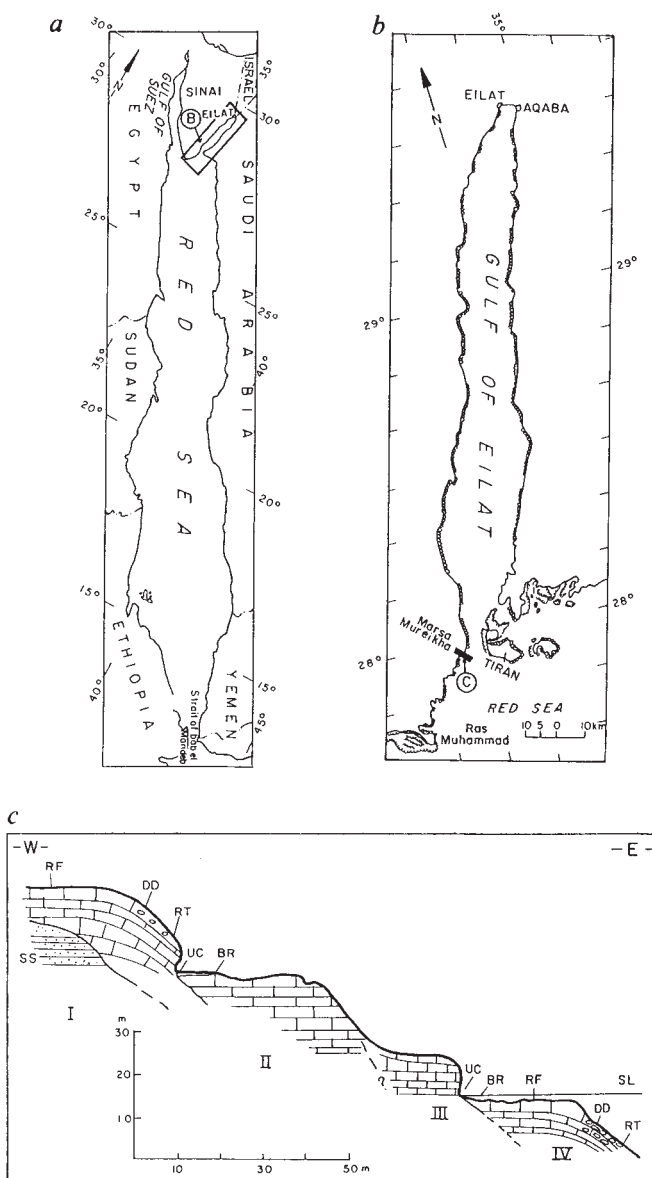


FIG. 1 Location map of a, the Red Sea; b, the Gulf of Eilat and its belt of fringing reefs; and c, a cross-section of the elevated late Quaternary fossil reefs, along the coastal belt in Marsa Mureikha. The reefs form morphological terraces labelled I–IV, from the oldest to the youngest, respectively. BR, beach rock; DD, depositional dips; RF, reef flat; RT, reef talus; SL, sea level; SS, sandstone substrate; UC, fossil shoreline undercut (modified after ref. 12). W, west; E, east.

TABLE 1 Summary of relevant characteristics of modern and fossil Red Sea corals

	Modern	Fossils	
	Coral S21	Coral Fos4	Coral 1389
Location	Eilat	The Garden, Marsa el-At	Wadi Mureikha, northern bank
Longitude	34°56'34"	34°21'00"	34°23'18"
Latitude	29°31'29"	27°54'34"	27°57'00"
Morphological terrace	Modern	Middle	Lower
Analyses			
Fluorescence	Absent	Present	Present
Phosphorescence	Absent	Present	Absent
Rhomboids of low-Mg calcite*	Absent	Present	Absent
Humic acid† ($\mu\text{g g}^{-1}$) in fluorescent bands (mean $\pm$ s.d.)		220 $\pm$ 29 (n=7)	284 $\pm$ 32 (n=3)
Humic acid ($\mu\text{g g}^{-1}$) in adjacent non-fluorescent bands (mean $\pm$ s.d.)	10.2 $\pm$ 7.4‡ (n=3)	153 $\pm$ 11 (n=7)	226 $\pm$ 13 (n=3)
Per cent aragonite§ (mean $\pm$ s.d.)	100 $\pm$ 0 (n=4)	85.5 $\pm$ 7.5 (n=4)	90.0 $\pm$ 2 (n=3)
Sr ($\mu\text{g g}^{-1}$)	7,800	6,570	6,930

* Samples were run on an Etec autoscan scanning electron microscope fitted with a Link 290 energy dispersive system.

† High-performance liquid chromatography (HPLC) was according to ref. 13. Water for chemical analysis was purified by a Millipore system (Bedford, Massachusetts) and then doubly distilled from potassium permanganate. Powdered coral skeletal matter (~20 mg) was weighed into a 4-ml glass vial and after the addition of 500 μl water, 75 μl concentrated hydrochloric acid (Aristar grade; BDH, UK) was added slowly with gentle shaking to effect solution. The sample was adjusted to pH ~6 with ~40 μl sodium hydroxide solution (10 M; Aristar grade, BDH, UK) and the final volume adjusted to 800 μl with water. Fifty microlitres of sample was injected into the system.

‡ Mean of three randomly selected areas, because no fluorescent bands exist.

§ Using X-ray diffractometry on a Rigaku D-max CN 2155D5 powder diffractometer with monochromatic Cu K α radiation.

|| Strontium was analysed by dissolving ~50 mg coral skeletal matter in a minimal amount of concentrated hydrochloric acid and adjusting the final volume to 10 ml with water. Samples were directly aspirated into a SpectraScan V direct-current plasma emission spectrometer (Beckman, California).

that had only low levels of incorporated humic acid (Table 1). Results for individual fluorescent and non-fluorescent bands of the fossil coral Fos4 are illustrated in Fig. 2.

Most of the fossil corals that we investigated were phosphorescent. After irradiation with ultraviolet light, fossil corals glowed for tens of seconds, which is far longer than the modern corals. We examined the crystal fabric of a phosphorescent and non-phosphorescent fossil coral, and found rhomboids of low-Mg calcite present in the pores of the phosphorescent coral (Table 1). The trace-element record supports this finding in that there are low amounts of magnesium in the inner part of the phosphorescent coral, which are not detected in the non-phosphorescent coral. The mineral composition of the corals by X-ray diffractometry (Table 1) indicates that the fossil corals underwent only initial stages of diagenesis, in which 85–90% of the aragonite was not replaced. Sample Fos4 underwent initial void-filling of low-Mg calcite, whereas sample 1389 had initial replacement of the aragonitic skeleton. The Sr content, from direct-current plasma spectrometry (Table 1), reconfirms our assertion that the fossil corals were not significantly affected by diagenesis, because the Sr content (in the range of 6,900–7,800 $\mu\text{g g}^{-1}$) is still typical of aragonitic corals^{14,15}. These results exclude any argument that the fluorescent compounds might have been introduced into the fossil corals during diagenesis.

Finally, we investigated the possibility of post-growth humic acid intrusion into the fossil corals by testing whether there had been inward migration of humic acid, in which case the humic acid concentration would be expected to be highest in the outer portions of the coral, with a decreasing inward gradient. Eight sub-samples of skeletal material along a horizontal transect in the middle of Fos4 were analysed for humic acid. Values were 159, 101, 106, 160, 210, 116, 107 and 143 $\mu\text{g g}^{-1}$ and represent the order in which the sub-samples were taken; the first and last indicated the two external portions of the coral (X and Y in Fig. 2). As these results show, there is no gradient in humic acid concentration along the transect. Furthermore, the dissolved humic acid concentration in soils is normally lower than in the coral skeletons. The concentration gradient between the coral head and surrounding soil would preclude humic acid intrusion (M.S., K. G. Boto and P. J. Isdale, manuscript in preparation). The absence of a humic acid gradient in the coral is consistent with this observation. Therefore, we propose that there are two independent phenomena: (1) fluorescent bands exist as a function of periodic events during the life of the corals, irrespective of later events; and (2) phosphorescence is attributable to events after the death of the corals, probably as a consequence of deposition of low-Mg calcite in the coral voids.

We attribute the increased concentrations of the terrestrially derived humic compounds in the fossil coral samples to the late Quaternary runoffs. The terrestrially derived fluorescence overlies a faint blue background fluorescence throughout the coral, probably as a result of the incorporation of marine humic acid⁶ and terrestrial residuals of the wet season humics (M.S., K. G. Boto and P. J. Isdale, manuscript in preparation). This explains the relatively high humic acid concentrations characterizing the non-fluorescent bands of the fossil corals (Table 1). Both the Sinai fossil corals and modern inshore Great Barrier Reef corals contain similar concentrations of humic acid in the non-fluorescent regions, but these concentrations are an order of magnitude greater than the humic acid concentrations in modern Sinai corals. Assuming that sea water contains at least some terrestrial

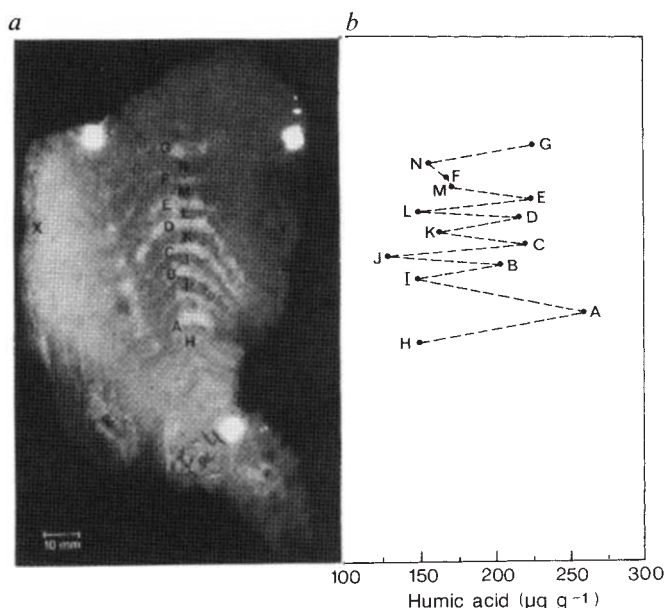


FIG. 2 a, Alternation of fluorescent (light) and non-fluorescent (dark) bands in fossil *Porites* (Fos4) under ultraviolet light. The fluorescent bands are superimposed on the low-density sub-bands. b, Humic acid concentrations from fluorescent (A–G) and non-fluorescent (H–N) bands. Samples for HPLC were taken from the centre of each band. The labelling in b corresponds to that in a, indicating the specific band from which each sample was taken. The points X and Y represent the horizontal transect in which the sub-samples for humic acid analysis were taken (see text).

humic acid even after periods of little or no terrestrial runoff, then the runoff in the Sinai during the late Quaternary was more like present-day runoff in the Great Barrier Reef than present-day runoff in the Sinai.

Finally, comparison of skeletal density bands (as shown by X-rays) and photographs of fluorescing fossils under ultraviolet light show that the fluorescent bands correspond to the low-density portions of the skeleton. Analysis of modern *Porites* corals from the living reef of the Gulf of Eilat showed that wide low-density bands were deposited during summer, and narrow high-density bands during winter³. The fossil corals have a similar density-banding pattern (wide low-density and narrow high-density bands). Assuming unchanged seasonal deposition of bands, the fluorescent bands in the fossil corals therefore correspond to summer events.

Our findings indicate that during the time of reef growth and high-stand sea levels, a wet climate, possibly with a summer rainfall regime, probably prevailed in the Sinai, in contrast to the present extremely dry conditions (with rare precipitation during winter). Most of the palaeoclimate evidence known for Israel and the Sinai regions extends to the periods of the Holocene and the last Glacial. The existence of a series of palaeosols in the present-day desert region of southern Israel (the northern Negev Desert) indicates that there were wetter climates corresponding to the global warmer periods of the last Glacial¹⁶⁻¹⁷. Pollen spectra and the isotopic composition of fossil groundwater¹⁸⁻¹⁹ suggest that during the late Würmian and mid Holocene, Israel experienced at least some summer rains. Other evidence for wetter conditions in the Negev Desert of Israel in the mid Holocene is indicated by a palaeodiet study of fossil land snails²⁰. Study of lake deposits extend our palaeoclimate knowledge of the region to the interglacial intervals of the isotope stages 5 and 7 (ref. 21), suggesting that during these warmer global episodes the arid southern region of Israel (Arava Rift Valley) was considerably wetter than it is now. Palaeoclimate oscillations, ranging from tropical humid to warm arid climates, in regions such as central Africa, the Ethiopian Plateau and eastern Sahara, were recorded from deposition of heavy minerals in the Nile delta during periods of the last Glacial and the mid Holocene²².

Several studies discuss monsoonal appearance in response to orbital insolation. Marine sapropels, deposited in the eastern Mediterranean sea as a result of heavy discharge from the Nile River, indicate that African monsoons were always at their heaviest during all the interglacials in the past 464 kyr as an immediate response to orbital variations of insolation²³. Climatic characteristics of the interglacial intervals, as detected from a 150-kyr oxygen-isotope pollen record from the Arabian Sea, describe the intensification of the summer southwest monsoonal flow²⁴⁻²⁵. Finally, climate models²⁶⁻²⁷ have been used to explain the monsoonal variability over the past 150 kyr. These models reveal that, under interglacial conditions, increased Northern Hemisphere solar radiation produced a larger land-ocean pressure gradient, strong winds and great precipitation over southern Asia and North Africa.

Our interpretation of summer rainfall in the Sinai desert, during the late Quaternary periods of high-sea-level interglacial intervals (1) supports climate evidence and models that show the linkage between monsoonal flow, interglacial intervals and orbital insolation; (2) provides possible evidence of northward extension of the monsoonal belt over the Sinai region and (3) extends the knowledge of the climate conditions prevailing in the Sinai more extensively than periods of the last Glacial. Analysis of fluorescent bands in fossil corals provides a new and promising method for palaeoclimate reconstruction. □

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Ascending and descending fluxes of lipid compounds in North Atlantic and North Pacific abyssal waters

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TWO classes of particles are generally recognized in oceanic waters, suspended and sinking. Previously, we demonstrated that there is also an upward flux¹. This represents a small proportion of the descending material but it is mostly composed of organic-rich particulate matter. A subsequent study² confirmed these results providing quantitative data on the ascending organic carbon and nitrogen. Based on sediment-trap experiments, we show here that these upward fluxes determine the biogeochemical cycling of some lipids in abyssal waters. Up-to-down flux ratios ranging between 0.19-190% and 15-13,000% have been found for the sterols and fatty acids, respectively. The ascending organic material corresponds essentially to zooplankton and crustacean debris, whereas algal patterns dominate in the settling particles. Our results indicate that formation of buoyant matter in abyssal waters may act as a selection mechanism by which some lipid components are recycled back to the upper water levels whereas others keep descending towards the bottom. This previously unreported process has implications for the interpretation of the lipid record in oceanic sediments, especially when molecular stratigraphy is used for palaeoenvironmental assessment³.

We concurrently sampled ascending and descending particulate matter at two stations over a period of one year. The North Atlantic site (E) is located at 32°46.2' N, 70°47.4' W over the

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TABLE 1 Sampling locations, mineralogy and flux of passively sinking and rising particles, organic carbon, fatty acids and sterols

Collection	Altitude			Mineralogy				Fluxes			
	Time (d)	Depth (m)	above sea floor (m)	Trap	CaCO ₃ (%)	Opal (%)	Al (%)	Total particles (mg cm ⁻² yr ⁻¹)	Organic carbon (μg cm ⁻² yr ⁻¹)	Fatty acids† (ng cm ⁻² yr ⁻¹)	Sterols† (ng cm ⁻² yr ⁻¹)
Station E (32°46.2' N, 70°47.4' W)											
Spring/Summer (1983)											
6 May–12 August	98	2,835	2,629	Upright	60.3↓	9.4↓	1.3↓	1.6↓	36↓	9.1↓	78↓
6 May–12 August	98	2,865	2,599	Inverted				0.0026† (0.16)*	2.5†(6.9)	1.4†(15)	0.36†(0.46)
6 May–12 August	98	4,685	779	Upright	56.8↓	8.2↓	1.8↓	1.8↓	91↓2.7↓	48↓	
6 May–12 August	98	4,730	734	Inverted				0.008† (0.44)	7.6† (8.4)	5.6† (210)	0.96† (2.0)
Summer/Autumn (1983)											
12 August–18 November	98	4,685	779	Upright	57.9↓	7.4↓	2.3↓	1.3↓	53↓	0.7↓	19↓
12 August–18 November	98	4,730	734	Inverted				0.0082† (0.63)	7.2† (14)	3.9† (580)	0.03† (0.16)
Autumn 1983/Winter 1984											
18 November–24 February	98	2,835	2,629	Upright	69.3↓	8.6↓	1.0↓	0.87↓	42↓	1.6↓	29↓
18 November–24 February	98	2,865	2,599	Inverted				0.009† (1.0)	7.2† (17)	4.2† (260)	6.3† (22)
Station W (39°29.4' N, 127°41.4' W)											
Autumn 1983											
9 September–15 November	67	1,235	2,995	Upright	29.6↓	28.5↓	1.8↓	1.3↓	93↓	31↓	110↓
9 September–15 November	67	3,785	445	Upright	19.3↓	16.5↓	5.1↓	3.0↓	95↓	2.5↓	15↓
9 September–15 November	67	3,815	415	Inverted				0.011† (0.37)	10† (11)	43† (1,700)	29† (190)
Autumn 1983/Winter 1984											
15 November–2 March	107	1,235	2,995	Upright	19.1↓	48.2↓	2.0↓	1.0↓	52↓	18↓	83↓
Summer 1984											
6 June–6 September	92	3,785	445	Upright	22.1↓	21.7↓	4.1↓	1.5↓	56↓	1.4↓	21↓
6 June–6 September	92	3,815	415	Inverted				0.0165† (1.1)	14† (25)	180† (13,000)	0.16† (0.76)

Conical sediments traps, developed from the basic design of Soutar *et al.*⁴, were moored in upright and inverted positions^{5–8}. They consist of a single fibreglass cone with a collection surface of 0.5 m² and a height-to-diameter ratio of 2:1. They were covered with 5-cm-deep nylon-fibre honeycomb baffles having 1-cm² cell openings. Their absolute collection accuracy was evaluated >60% from Th isotope measurements^{5–8}. The trapped material was centrifuged, rinsed free of salt, freeze-dried and extracted with (2:1) dichloromethane-methanol. The concentrated extracts were esterified with diazomethane and silylated. Gas chromatography (GC) was performed with a Carlo Erba 4160 instrument equipped with FID (330 °C), on-column injection and a 25 m × 0.32 mm i.d. column coated with CP-Sil 8 CB (60–300 °C at 4 °C min⁻¹). Hydrogen was the carrier gas. GC coupled to mass spectrometry (GC-MS) was carried out with a Hewlett-Packard 5995 instrument equipped with an HP 300 data system. The GC traces were quantitated using an external standard of silylated cholest-5-en-3β-ol and 24-ethylcholest-5-en-3β-ol, and methyl *n*-heptacosanoate and *n*-docosanoate. Samples and standards were repeatedly injected until <5% dispersion was observed. Sample aliquots were derivatized with dimethyl disulphide for determination of the double-bond position on fatty acids by GC-MS.

* Per cent relative to downward flux collected at the same time and roughly the same depth.

† Data obtained by summation of the corresponding GC peaks.

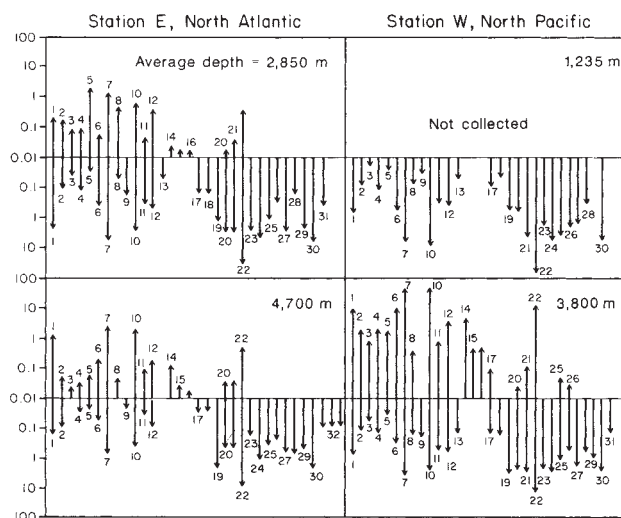
Hatteras Abyssal Plain, ~350 km from shore. The North Pacific site (W) is located at 39°29.4' N, 127°41.4' W within the California current, ~300 km offshore.

Table 1 shows dates, deployment times and depths of the upright and inverted traps included in this study, as well as brief descriptions of the trap design, sample processing techniques and analytical methods^{4–8}. The mineralogy and measured fluxes of total particles (TPF), organic carbon (OCF), and total fatty acids and sterols, the major lipid fractions, are also given in Table 1. The downward particle flux from both sites is dominated by biogenic debris. In the Atlantic the settling flux is rich (57–69%) in carbonate shell material from foraminifera and calcareous nannoplankton and contains 7–9% opal from diatom and radiolarian tests (Table 1). The settling flux in the Pacific contains less carbonate (19–30%) but more opal (17–48%, Table 1). The buoyant-particle flux is dominated by spherical globules, which could be egg forms, and oils, which form a surface film on the recovered samples. The downward TPF and OCF are comparable to others observed in similar depths at central sites of the Pacific^{1,2,9} and Atlantic¹⁰ Oceans. The absence of an OCF decrease with depth is consistent with the known low degradation of sinking biogenic debris below ~1,000 m (ref. 11). The upward fluxes are also consistent with previous data^{1,2}. The ascending particles are a small fraction of the sinking material

but in terms of OCF they represent between 7 and 25%. This up-to-down flux ratio is even higher for the lipid fractions, namely the fatty acids (15–13,000%). The higher content of organic carbon, and especially lipids, in the ascending material point to a *de novo* biosynthetic origin. This can be investigated from the molecular composition of the lipids, which is represented in Fig. 1 in the form of average ascending and descending fluxes. For a general overview, Fig. 2 shows examples of the gas chromatographic profiles of the total lipids from an upward and downward facing trap.

Sterols predominate in the descending particulate matter. Cholest-5-en-3β-ol is the main component. It occurs with 27-nor-24-methylcholesta-5,22-dien-3β-ol, cholesta-5,22-dien-3β-ol, 24-methylcholesta-5,22-dien-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol and 24-methylcholest-5-en-3β-ol, a mixture generally characteristic of diatoms¹². The sterol 4α,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol is indicative of dinoflagellates¹³. The presence of these sterols related to primary producers in the descending particles is consistent with the presence of phytol, the side-chain decomposition product of chlorophyll¹⁴. The sterol 24-ethylcholest-5-en-3β-ol may originate from both algal or higher-plant sources¹². The presence of higher-plant detritus in the upright trap material is more specifically represented by dehydroabietic acid^{15,16}. Another significant steroidal

FIG. 1 Average (~ 180 d) ascending ($\uparrow$) and descending ($\downarrow$) fluxes ($\text{ng cm}^{-2} \text{yr}^{-1}$) of the main lipids identified in the particles collected with inverted ($\uparrow$) and upright ($\downarrow$) traps moored at North Atlantic and North Pacific stations. Note that the vertical scales are logarithmic. Arrow identifications are as follows: (1) *n*-tetradecanoic acid; (2) *iso*-pentadecanoic acid; (3) *anteiso*-pentadecanoic acid; (4) *n*-pentadecanoic acid; (5) *n*-hexadecanol; (6) *n*-hexadec-9-enoic acid; (7) *n*-hexadecanoic acid; (8) *n*-octadecanol; (9) phytol; (10) *n*-octadec-9-enoic acid; (11) *n*-octadec-11-enoic acid; (12) *n*-octadecanoic acid; (13) dehydroabiatic acid; (14) *n*-eicosenoic acids (11- and 13-en); (15) *n*-eicosanoic acid; (16) *n*-docosenoic acids (11- and 13-en); (17) *n*-docosanoic acid; (18) *n*-tetracosanoic acid; (19) 22,29,30-trisnorhopan-21-one; (20) 27-nor-24-methylcholesta-5,22-dien-3 β -ol; (21) cholesta-5,22-dien-3 β -ol; (22) cholest-5-en-3 β -ol; (23) cholest-4-en-3-one; (24) 24-methylcholesta-5,22-dien-3 β -ol; (25) 24-methylcholesta-5,24(28)-dien-3 β -ol; (26) 24-methylcholesta-5-en-3 β -ol; (27) 24-ethylcholesta-5-en-3 β -ol; (28) 4 α ,23,24-trimethyl-5 α (H)-cholest-22-en-3 β -ol; (29) *n*-triacontan-15-on-1-ol; (30) *n*-triacontan-1,15-diol; (31) homohopan-31-ol; (32) bishomohopan-32-ol; (33) trishomohopan-33-ol. Detailed information on the samples included in this study is given in Table 1. Ascending particles at the North Pacific station were not collected for the 1,235-m level.



component is cholest-4-en-3-one, a microbial transformation product of sterols that is usually the major steroidal ketone of deep-water particulate matter^{10,17}.

Fatty acids, the other major group of sinking particulate lipids, constitute a mixture dominated by *n*-hexadecanoic acid, with important amounts of *n*-tetradecanoic, *n*-hexadec-9-enoic, *n*-octadec-9-enoic and *n*-octadecanoic acids. This distribution corresponds to contributions from phytoplankton and zooplankton (for example, *n*-tetradecanoic, *n*-hexadecanoic and hexadec-9-enoic acids are found in diatoms)¹⁸. Some fatty acids present as minor components, that is, *iso*- and *anteiso*-pentadecanoic acids and octadec-11-enoic acid, are characteristic of microbial sources.

Microbial inputs may also account for the occurrence of 22,29,30-trisnorhopan-21-one, the hopanoid component that has been found in other deep-water trap experiments¹⁹. However, this hopanone may also originate from cyanobacterial inputs. These are also represented by a series of C₃₁-C₃₃ hopanols, *n*-triacontan-15-on-1-ol and *n*-triacontan-1,15-diol²⁰. The importance of bacterial and cyanobacterial aggregates in the deep sea has recently been demonstrated²¹.

Fatty acids predominate in the ascending particulate material (Fig. 1). They consist of a mixture of C₁₄-C₂₂ homologues, with

n-tetradecanoic, *n*-hexadecanoic and *n*-octadec-9-enoic acids as major components. Unsaturated acids are present in high proportion. Among these the *n*-eicosenoic and *n*-docosenoic acids are very significant because they do not occur in the sinking material. The second major lipid group in the ascending particles is composed of the sterols which constitute very simple mixtures: high predominance of cholest-5-en-3 β -ol, with 27-nor-24-methylcholesta-5,22-dien-3 β -ol and cholesta-5,22-dien-3 β -ol as minor components. At station W (3,800 m depth) small amounts of 24-methylcholesta-5,24(28)-dien-3 β -ol and 24-methylcholesta-5-en-3 β -ol are found. These distributions of fatty acids and sterols are characteristic of zooplankton²²⁻²⁴ or other crustacea (amphipods)²⁵. The buoyant properties of wax esters, triglycerides and other lipids isolated from such animals are known^{26,27}. Accordingly, most of the ascending organic material may be derived from their metabolism (excretion, reproduction, decay and so on).

The strong contrast between the composition of the sinking and buoyant particulate matter makes it unlikely that resuspended material contributed significantly to the inverted traps. No terrigenous biomarkers have been detected among the ascending particles. Furthermore, the lack of phytol, hopanols, hopanones, *n*-triacontan-15-on-1-ol and *n*-triacontan-1,15-diol indicates

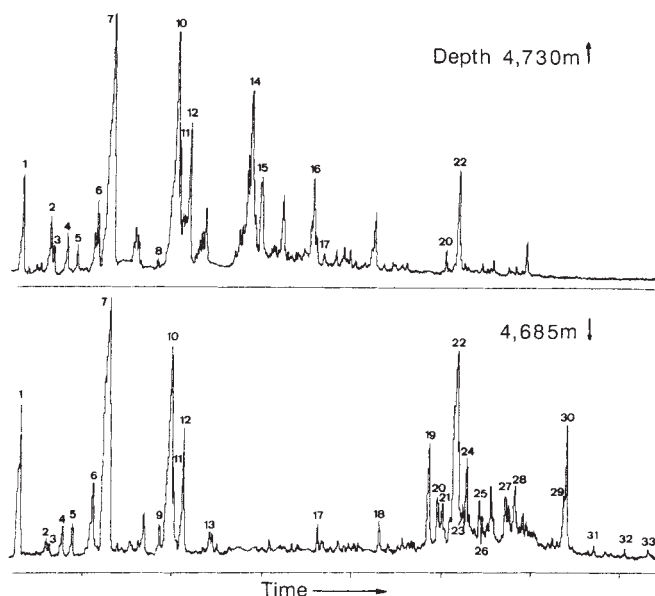


FIG. 2 Gas chromatographic profiles of the lipid compositions of two representative samples of passively sinking and rising particles collected at station E (Atlantic Ocean) between 12 August and 18 November (1983). Depths: 4,685 m ($\downarrow$) and 4,730 m ($\uparrow$). Peak identifications as in Fig. 1.

that neither cyanobacteria nor other primary producers contribute significantly to the rising material.

The planktotrophic activity of deep-water zooplankton and benthic crustacea is well known²⁸. The ability of these organisms to incorporate fatty acids and alcohols from algal particulate matter into their wax esters has also been demonstrated^{19,29}. They are even able to incorporate fatty acids dissolved in sea water³⁰. Thus, these animals probably consume a substantial fraction of the organic carbon arriving at the abyssal waters in the form of fecal pellets^{31,32}. Besides the commonly accepted degradation mechanisms of particulate organic matter in the ocean (such as oxidation, microbial attack), ingestion by deep-water crustacea and other zooplankton can be another important way for the transformation and recycling of this material. This should not necessarily result from direct intake. Heterotrophic degradation (either by microorganisms, zooplankton or micronekton) involves the incorporation of sinking organic carbon into the standing stock of suspended particulate matter³³. Zooplankton ingestion of the suspended lipids may also yield buoyant materials. In this respect, a recent comparison between the lipid composition of settling and suspended particles at ~1,500 m depth has shown that the latter are largely enriched in fatty acids³⁴, which is consistent with the compositional differences between settling and ascending particles observed here.

The overall process leads to a selective distribution where labile organic compounds are returned to shallower waters in the form of buoyant particles, whereas only the more refractory organic matter passes to the bottom. Besides reproductive materials (for example, eggs), buoyant particles may derive from lipids of lysed cells and disintegrating fecal pellets.

Buoyancy is faster than normal diffusion or mixing processes. On a global scale these ascending particles may represent an important pathway in the biogeochemical cycles of nutrients, organic matter and perhaps metals or pollutants. In addition, this turnover of labile lipids may strongly diminish the flux of some components to the sediments. This must be considered when biomarkers are used for the investigation of the sedimentary record, especially if palaeoenvironmental assessment is intended. Thus, the ultimate deposition rate of many compounds, and therefore the sedimentary biomarker pattern, may be influenced by the upward-to-downward flux ratios. The combined effects of these fluxes determine a selective enrichment of sedimentary higher-plant molecules with respect to the proportion of terrigenous components in the surface water particulates. For algal lipids, the mid-water up-to-down ratios represent a factor of quantitative variation of the same order as changes in the processes of the euphotic zone or post-depositional reactions. □

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An oxygen isotope discontinuity in high-grade rocks of the East Humboldt Range, Nevada

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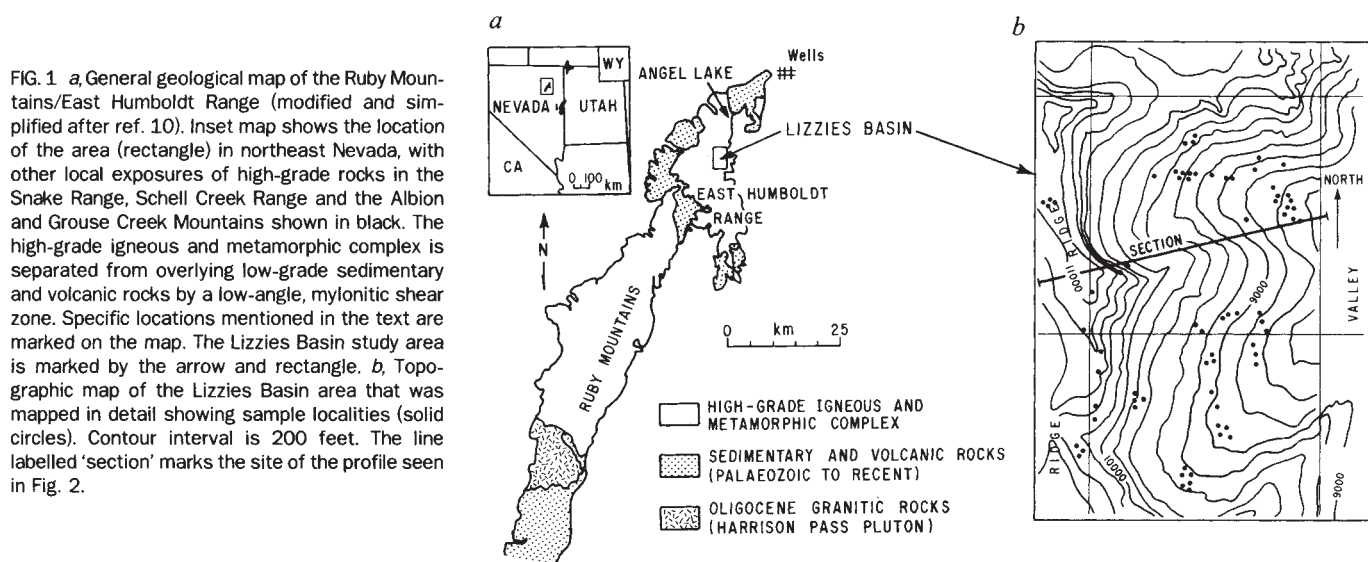
RECENT studies of metamorphic terranes document sharply contrasting $^{18}\text{O}/^{16}\text{O}$ systematics¹. Some regions are isotopically homogeneous, but with decreased $^{18}\text{O}/^{16}\text{O}$ ratios (see, for example, refs 2–4), whereas others preserve steep gradients in oxygen isotope composition^{5–7}. These results indicate that some terranes experience very large, pervasive fluid fluxes, whereas others may, at least locally, remain virtually fluid-absent. Here we report a very detailed oxygen isotope study of a high-grade metamorphic terrane, revealing striking systematic variations that are closely correlated with structural depth. In particular, metamorphic rocks that are low in ^{18}O , first detected at deep structural levels in a preliminary study of the area⁸, are now shown to be restricted to a clearly defined zone several hundred metres thick and extending for at least 3 km laterally. The most striking feature is the existence of a very sharp oxygen isotope discontinuity marking the upper boundary of the low- ^{18}O zone and separating it from an isotopically heavier and more heterogeneous (although still high-grade) overlying region.

In the Ruby Mountains/East Humboldt Range of north-eastern Nevada, a thick sequence of amphibolite facies meta-sedimentary and igneous gneisses and intrusive granitoids is overlain by a cover of low-grade, faulted Upper Palaeozoic to Tertiary rocks^{9–11} (Fig. 1). The metasediments are mostly metamorphosed Palaeozoic and Upper Pre-Cambrian sedimentary rocks from the miogeoclinal sequence exposed regionally throughout the eastern Great Basin. Igneous activity and associated metamorphism range from the Jurassic to the mid-Tertiary^{12–14}. The thermal and tectonic history during the Mesozoic is poorly understood, but the Tertiary events seem to be mostly due to regional extension, commencing in the Eocene and continuing episodically to the present¹¹.

The high-grade rocks include quartzite, marble, calc-silicate gneiss, pelite, psammite, leucogranite, biotite monzogranite, hornblende diorite, quartz-feldspathic orthogneiss and amphibolite. These comprise a complex, interlayered and structurally transposed sequence of many hundreds of rather thin (centimetres to tens of metres) gently dipping units (Fig. 2), the morphology and attitude of which reflect strong attenuation processes¹⁵. About 4 km² were mapped in detail at a scale of 1:10,000 (Fig. 1); a section through this region is depicted in Fig. 2. Although complete cartographic representation on this scale is impossible, Fig. 2 gives a good indication of the relative proportion of the different lithologies and their spatial distribution. No mappable isograds have yet been identified, but pelites show textural evidence for extensive partial melting, implying that the entire section of Fig. 2 exceeded 600 °C (and much of it probably 700 °C) (ref. 16). Locally, the proportion of granitoid over 50 m of section can be as high as 80%, but more typically

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it is 50% or less in the Upper Zone, and 70% or less in the Lower Zone.

At Angel Lake, 10 km to the north of Lizzies Basin (Fig. 1), a monzogranite sill has been dated by the U-Pb zircon method at 29 Myr (ref. 13). No reliable age has been obtained for the leucogranite pods, although some cross-cut (and therefore post-date) monzogranites. Several relatively thick sills of hornblende diorite occur at high structural levels in the Lizzies Basin area (Fig. 2), but the only other mafic rocks are sporadic tiny pods that make up less than 1% of the overall section.

Of all preliminary $^{18}\text{O}/^{16}\text{O}$ analyses from the East Humboldt Range⁸, samples from Lizzies Basin were found to have exceptionally low $\delta^{18}\text{O}$ values in comparison with normal metamorphic rocks and crustally derived granites (Fig. 3). These samples are from the deepest structural levels exposed in the whole range, and analyses of individual mineral phases show that these rocks are in high-temperature (that is, above 600 °C) oxygen isotope equilibrium. Note also that quartz from leucogranites, pelites and calc-silicate gneisses varies less than 0.5% in seven samples, indicating very thorough equilibration in these contrasting rock-types. To establish the extent of the zone of low- ^{18}O metamorphic and igneous rocks at Lizzies Basin, we analysed about 80 samples from the mapped area (Fig. 1). Results are shown in Fig. 4, plotted against topographic altitude of the samples; because lithological layering is almost horizontal, there is a close correspondence between the layers shown in Fig. 2 and the layers (and altitudes) from which the samples shown in Fig. 4 were taken.

There is a very sharp discontinuity in the data at ~9,000 feet. All silicate samples from below this level have a narrow range of $\delta^{18}\text{O}$ between +6.2 and +8.4; carbonates range between +9.2 and +11.7. Overall, the mean $\delta^{18}\text{O}$ below 9,000 feet (weighted for lithological proportions) is about +7.6. Above this level, the weighted mean of all samples is about +10.4. The proportions of the various lithologies are similar at all levels, except that there is little quartzite in the lower part of the section, less carbonate and a higher proportion of granite, particularly leucogranite. Note that the discontinuity also shows up in individual lithologies, such as the carbonates or pelites. Detailed mapping demonstrates that the discontinuity is not marked by any obvious structural feature such as a fault^{15,17}. The $\delta^{18}\text{O}$ values for the Lower Zone are also much more homogeneous than those in the Upper Zone, implying that internal equilibration was more effective. Thus transport of oxygen within and between all of the lithologies was much more important in the Lower Zone, and transport across the discontinuity was limited.

Oxygen transport was almost certainly promoted by fluid infiltration, which is probably reflected by the abundant quartz-biotite and calcite-calc-silicate veins observed in Lower Zone rocks at Lizzies Basin¹⁶.

Another important feature of the data set is the contrast in the mean $\delta^{18}\text{O}$ value in the Upper and Lower Zones. Hence the systematics cannot simply be explained by internal homogenization of $^{18}\text{O}/^{16}\text{O}$ ratios, because this would result in similar mean values in both the Upper and Lower Zones. At least one zone, and probably both, have exchanged with an external oxygen reservoir with a contrasting isotope composition. This point may be inferred simply by observing that most of the metasedimentary lithologies have much lower $\delta^{18}\text{O}$ values than

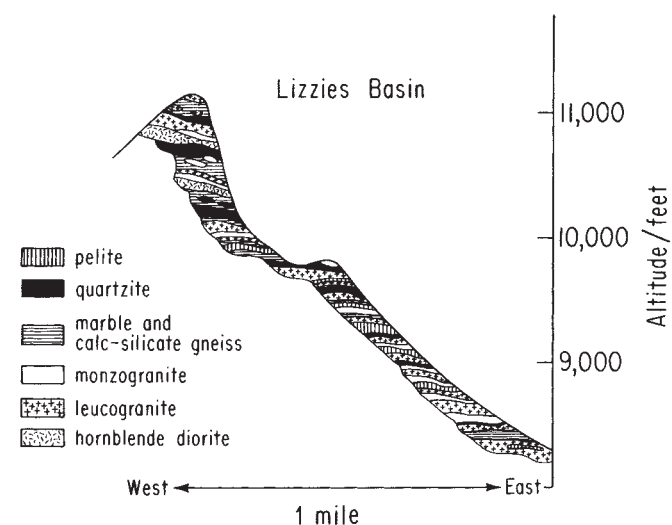


FIG. 2 Geological cross-section through the Lizzies Basin region (see Fig. 1) showing the proportion and distribution of lithologies, drawn along the line shown in Fig. 1b. Note that the proportion of granite at deeper structural levels increases, but that the same rock types persist throughout the section. Although there is a continuum of compositions between quartzite and pelite corresponding to a wide range of quartz contents, intermediate rocks (normally termed psammites) are grouped here as either quartzites or pelites. Sillimanite is widespread in pelites, but garnet is relatively rare. Marbles are defined as containing >80% calcite with minor quartz, feldspar and calc-silicate minerals. Calc-silicate gneisses contain very little or no calcite and comprise mostly calc-silicate minerals.

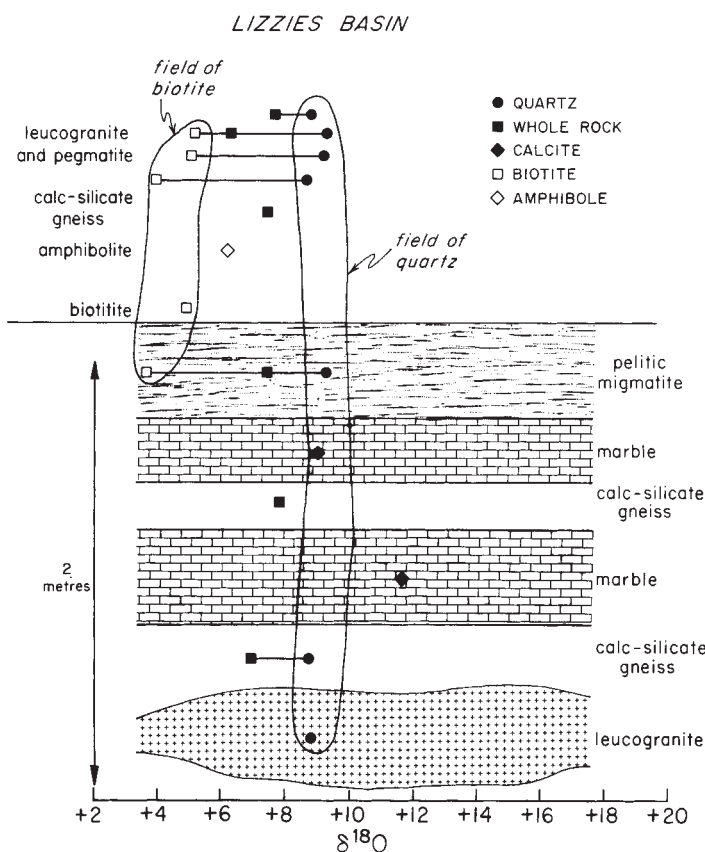
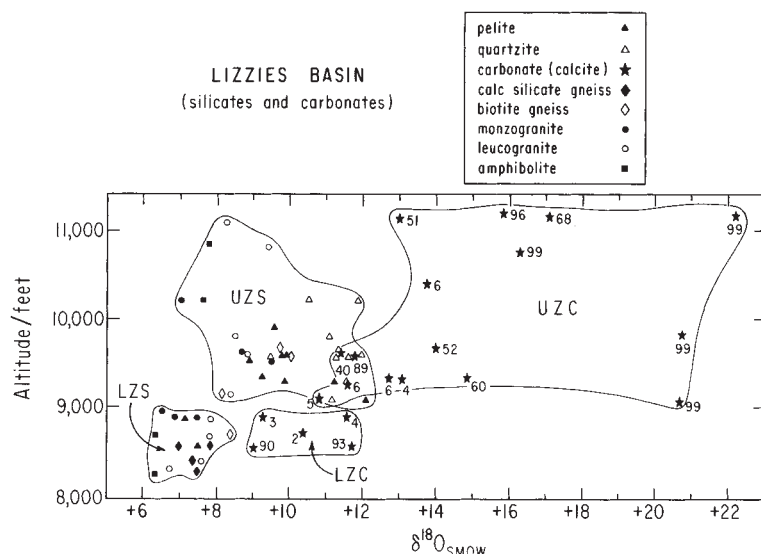


FIG. 3 Oxygen isotope whole-rock and mineral data⁸ from Lizzies Basin, including samples from a 2-m section through interlayered metasediments and granites. The upper part of the diagram shows other data, listed by lithology, for samples collected nearby (over an area of ~0.5 km²). Note the extremely narrow range shown by $\delta^{18}\text{O}$ of quartz and biotite samples from a variety of contrasting rock types; quartz-biotite fractionations of 4–5‰ are typical of high-grade metamorphic rocks, indicating that all these samples are well equilibrated. The two calcite samples are slightly out of equilibrium with the quartz and biotite (see Fig. 4), but are nevertheless very ^{18}O -poor compared with their sedimentary protoliths and have undergone $\delta^{18}\text{O}$ shifts in excess of 10‰. $\delta^{18}\text{O} = [(^{18}\text{O}/^{16}\text{O})_{\text{sample}} / (^{18}\text{O}/^{16}\text{O})_{\text{standard}}] - 1$, where the standard is standard mean ocean water (SMOW).

FIG. 4 Whole-rock $^{18}\text{O}/^{16}\text{O}$ data for silicate rocks and also calcite from carbonate rocks (stars) from the Lizzies Basin area plotted according to their altitude (see text, and compare with Fig. 2). The numbers next to the carbonate data points denote the weight percentage carbonate in each sample. 'Amphibolite' includes samples from the hornblende diorite sills at high structural levels (see Fig. 2), as well as from tiny mafic pods at deeper levels in the section. In the Lower Zone, both calcite-rich and calcite-poor samples have been extensively ^{18}O depleted, but in the Upper Zone, some of the very calcite-rich samples have been unaffected. Note the contrast between the very homogeneous Lower Zone (particularly notable for silicates) and the overlying, much more heterogeneous Upper Zone, with the discontinuity between them at ~9,000 feet. The calcite marbles are the only Lower Zone lithologies that have not been isotopically homogenized (see also Fig. 3). LZS, Lower Zone silicates; LZC, Lower Zone carbonates; UZS, Upper Zone silicates; UZC, Upper Zone carbonates.



normal sediments. Pelites within the Upper Zone range from +12.1 to +8.9, which is substantially lower than the typical $\delta^{18}\text{O}$ range of shales (+14 to +16), and many carbonates have also been strongly ^{18}O -depleted. In the Lower Zone, ^{18}O -depletion is even more extreme in all rock types. Interestingly, quartzite and pure calcite marbles (but not calcite-poor marbles or calc-silicate gneisses) show the least depletion of ^{18}O in both the Upper and Lower Zones. Thus, the highly refractory, unreactive and relatively plastic (at high temperatures) pure quartzites and carbonates were probably least permeable to metamorphic fluids.

Igneous rock types generally have 'normal' $\delta^{18}\text{O}$ values (between +6 and +12), although leucogranites have unusually low values, considering that they are probably largely derived from metasediment. They are compositionally and texturally similar to leucogranites in the Northern Ruby Mountains¹², which commonly have $\delta^{18}\text{O}$ in excess of +12. If the leucogranites at Lizzies Basin had a similar origin, then they or their sources must have also been strongly ^{18}O -depleted. Mafic igneous rocks are sparse, but those that occur have primitive igneous $\delta^{18}\text{O}$ values close to +6 in both zones. This is important because it indicates that these rocks have not become ^{18}O -enriched as the metasediments became ^{18}O -depleted, thus ruling out local oxygen isotope homogenization as a cause of low $\delta^{18}\text{O}$ values in the metasediments.

A sharp boundary in oxygen isotope composition, recognizable on a regional scale and traceable laterally for several kilometres, has not been observed previously in other metamorphic sequences. This may be because earlier studies were not so detailed, nor terranes so well exposed, as in this case. Two plausible explanations exist, which need not be mutually exclusive. The boundary may represent a barrier to fluid flow, such as a low-permeability rock layer. This could provide a 'seal', which would cap a lower, possibly convective, circulation system and isolate it from the Upper Zone. Such a 'seal' has already been considered in models proposing that large-scale convective circulation of pore fluids commonly occurs within metamorphic terranes¹⁸, although the nature of the 'seal' was not specified. In this respect, it is interesting to note that a now semi-continuous pure calcite marble layer occurs close to the Upper/Lower Zone boundary, although its precise location relative to the boundary is still to be determined. Pure calcite marbles are the least exchanged, and therefore probably the least permeable, lithological layer in the Lizzies Basin sequence.

As such, a pure calcite layer represents the most plausible type of upper boundary layer to a convective system.

A second possibility is that the boundary represents an oxygen infiltration front, marking the upper limit of low- ^{18}O oxygen penetration. The isotope signatures of the various chemical components in an infiltrating fluid will be advected various distances that depend on their partitioning between rock and fluid^{19,20} and the resultant effect in the rocks will be to leave a series of abrupt isotope discontinuities, perhaps slightly broadened by diffusive processes. The boundary between the two zones at Lizzies Basin could reflect the position at which such a front was arrested.

The $^{18}\text{O}/^{16}\text{O}$ systematics at Lizzies Basin cannot be explained by simple isotope homogenization or mixing at the structural levels exposed today. The measured values show that the igneous rocks in the sequence, particularly the mafic ones, have not become ^{18}O -enriched by mixing with oxygen derived from the metasediments. Furthermore, simple material balance calculations indicate that the volumetric proportion of igneous rock in the Lizzies Basin sequence is insufficient to generate the ^{18}O -depletions in the sediments, even if they all initially had $\delta^{18}\text{O}$ of +6 (ref. 21). Because the Lower Zone values seem limited by the value +6, it is still tempting to infer that the values are being buffered by deep-seated mantle-derived rocks. In this respect, we note that large-scale Tertiary mafic intrusions have been invoked to explain regional tectonic, petrological and geophysical observations in this area²²⁻²⁴. If the Lizzies Basin rocks equilibrated with such a reservoir, this process must have been on a vast scale (possibly occurring over several episodes) with the Lower Zone (and perhaps, in part, the Upper Zone) equilibrating with a significant fraction of the total crustal thickness of isotopically primitive igneous rock to bring the $\delta^{18}\text{O}$ values down so close to +6. Equilibration must have been promoted by crustal-scale fluid or melt transport, perhaps driven by the emplacement of the same large volumes of basaltic magma within the middle or lower crust, in an analogous (though much more deep-seated) process to the hydrothermal circulation observed around shallowly emplaced layered intrusions²⁵.

Another possibility is that the rocks equilibrated with low- ^{18}O aqueous pore fluids, as has been indicated in other terranes⁴. In this case, the amount of fluid necessary might have been lower, because its $\delta^{18}\text{O}$ value could have been less than +6. Inasmuch as the lowest values are observed at the deepest structural levels, the fluid would apparently have had to be derived from below or adjacent to the present level of exposure, perhaps supplied from sedimentary layers emplaced at deep structural levels in the crust during earlier thickening events. A more accurate and quantitative definition of flow geometry and fluid sources awaits the results of research now in progress. □

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Thermoluminescence dating of a 50,000-year-old human occupation site in northern Australia

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THE oldest secure date for human occupation in Greater Australia is 40 kyr from eastern Papua New Guinea¹, whereas slightly younger dates have been reported from southern Australia². We now report thermoluminescence (TL) dates that suggest the arrival of people between 50 and 60 kyr in northern Australia. TL dates were obtained from sandy footslope deposits at two former occupation sites that yielded a range of stone artefacts in their primary depositional setting. Artefacts terminated mid-way down one profile, which had a basal age of about 100 kyr. Confidence in the TL dates is given by their close correspondence with radiocarbon dates obtained from the upper occupation levels. These TL dates are not only the oldest yet proposed for Aboriginal occupation but also may mark the time of initial human arrival on the Australian continent.

The two sites (Malakunanja II and Malangangerr) are located on low-gradient sand aprons developed at the foot of the western escarpment of the Arnhem Land plateau. Both sites have been excavated previously^{3,4}. The deposit at Malakunanja II comprises 4.6 m of poorly sorted medium sands overlying basal rubble. TL samples were collected from deposits adjacent to the original excavation by sand auger and subsequent excavation (Fig. 1, Table 1). A radiocarbon date (SUA-265) of $18,040 \pm 300$ yr BP had been obtained previously³ from 188-215 cm depth and a TL sample (KTL97) collected from a similar depth interval yielded an age of 24 ± 5 kyr. These dates are comparable given that the samples were collected on different occasions and from locations separated laterally by around 1 m. We collected charcoal from 146-147 cm depth, which gave an age (ANU-7006) of $13,390 \pm 400$ yr BP. This date agrees well with the TL date of 15 ± 3 kyr obtained for KTL165: most confidence is placed in this comparison as both samples were collected from a small depth interval, the latter from marginally deeper, during the same excavation. At Malangangerr, four radiocarbon dates (GaK-628, GaK-629, ANU-77 and ANU-19) have been reported previously⁴ which span the period 18-23 kyr BP. We collected three TL samples (KTL157, 125 and 126) from the adjacent deposits. KTL125 was collected from the same depth as the radiocarbon samples and yielded an age of 16 ± 5 kyr: this coincides, within the estimate of uncertainty, with the four radiocarbon dates.

More than 1,500 artefacts were recovered from the lowest occupation levels at Malakunanja II. These include silcrete flakes, pieces of dolerite and ground haematite, red and yellow ochres, a grindstone and a large number of amorphous artefacts made of quartzite or white quartz. There is a marked peak in artefact density from 2.3 to 2.5 m depth with occupation starting abruptly at 2.6 m depth. As we cannot exclude the possibility

TABLE 1 Thermoluminescence data, radionuclide activities and estimated ages

Sample number	Depth (cm)	^{238}U	^{226}Ra	^{210}Pb	^{228}Ra	^{228}Th	^{40}K	Total Dose rate*† (Gy kyr ⁻¹)	ED plateau§ (Gy)	Plateau range (°C)	TL Age (kyr)
Malakunanja II											
KTL156	1-2	—	—	—	—	—	—	0.90 ± (0.07, 0.05)*	1.2 ± 0.2	350-435	0.2 ± (1.2, 1.3)
KTL165	149-155	13.8 ± 3.1	12.2 ± 0.4	18.9 ± 6.5	22.4 ± 0.7	24.0 ± 0.5	54.2 ± 3.2	1.08 ± (0.09, 0.07)	17 ± 2	310-380	15 ± (2, 3)
KTL97	190-209	12.3 ± 3.1	12.0 ± 0.3	16.4 ± 6.2	20.9 ± 0.7	21.5 ± 0.4	40.3 ± 2.7	0.95 ± (0.08, 0.06)	24 ± 3	350-465	24 ± (4, 5)
KTL164	230-236	11.4 ± 3.8	10.7 ± 0.9	13.9 ± 3.2	18.7 ± 1.9	20.8 ± 0.8	46.1 ± 2.6	0.91 ± (0.05, 0.06)	42 ± 4	265-500	45 ± (6, 9)
KTL158	241-254	9.6 ± 2.9	10.1 ± 0.3	10 ± 5*	18.6 ± 0.6	18.8 ± 0.3	36.6 ± 2.5	0.79 ± (0.07, 0.05)	42 ± 4	270-410	52 ± (7, 11)
KTL162	254-259	9.3 ± 2.6	12.1 ± 0.3	8.3 ± 5.8	19.2 ± 0.6	19.9 ± 0.3	32.2 ± 2.4	0.77 ± (0.08, 0.05)	48 ± 5	270-500	61 ± (9, 13)
KTL141	295-315	12.8 ± 3.2	10.4 ± 0.3	11.1 ± 6.7	18.3 ± 0.7	19.6 ± 0.4	39.6 ± 2.9	0.84 ± (0.09, 0.05)	56 ± 6	355-485	65 ± (10, 14)
KTL116	390-411	15.1 ± 3.1	11.6 ± 0.3	11.7 ± 6.4	21.8 ± 0.7	23.4 ± 0.4	55.0 ± 3.1	0.97 ± (0.09, 0.06)	85 ± 9	345-410	86 ± (12, 18)
KTL163	452-458	13.1 ± 3.3	16.9 ± 0.4	14.8 ± 7.0	27.9 ± 0.8	29.6 ± 0.4	87.4 ± 4.1	1.22 ± (0.10, 0.08)	132 ± 13	325-500	107 ± (14, 21)
Malangangerr											
KTL157	1-2	—	—	—	—	—	—	0.90 ± (0.08, 0.05)*	1.5 ± 0.3	350-425	0.6 ± (1.2, 1.3)
KTL125	123-138	20.6 ± 5.3	18.3 ± 0.4	19.3 ± 7.4	25.5 ± 0.8	27.1 ± 0.4	19.1 ± 2.5	1.06 ± (0.10, 0.07)	18 ± 4	260-430	16 ± (4, 5)
KTL126	192-201	20.0 ± 6.1	16.9 ± 0.5	27.8 ± 8.6	28.3 ± 0.9	27.1 ± 0.5	23.0 ± 3.0	1.21 ± (0.12, 0.07)	39 ± 8	345-400	32 ± (7, 9)

* Values marked by an asterisk are estimated and assume secular equilibrium in the decay series.

† Mean ± random uncertainty. Concentrations of 1 p.p.m. ^{238}U , 1 p.p.m. ^{232}Th and 1% ^{40}K correspond to specific activities of 12.5, 4.1 and 31.7 Bq kg⁻¹ respectively.

‡ Mean ± (random uncertainty, systematic uncertainty). Soil moisture variations are included in the systematic uncertainty term: sample porosity was measured and the range encompassed by a mean value of 25 ± 5%. The fraction of burial time spent at saturation is estimated as 20 ± 20%; this value accommodates present-day conditions and variations due to long-term climatic change. A β attenuation factor of 0.93 ± 0.03 and a cosmic ray dose rate of 0.15 ± 0.025 Gy kyr⁻¹ for buried samples and 0.28 ± 0.05 Gy kyr⁻¹ for near-surface samples are adopted. The internal α activity of quartz, after HF acid etching, is estimated from the radionuclide activities (measured by γ -ray spectrometry) of each unetched sample, an α efficiency 'a' value of 0.10 ± 0.05, and an etched/unetched α count ratio of 0.035 ± 0.035: the latter value incorporates the ratios of 0.034 ± 0.006 and 0.019 ± 0.003 determined by thick-source α counting of unsealed extracts, before and after etching, for selected samples from Malakunanja II and Malangangerr respectively. The trivial dose rate contribution (~ 0.003 Gy kyr⁻¹) from ^{87}Rb is ignored.

§ Mean ± random uncertainty. Random uncertainties are estimated at 10% for the Malakunanja II samples to encompass the reproducibility (2-8%) among the naturals and the uncertainty (3-8%) in the growth curve fits at the 325 °C glow curve peak; it also reflects the 8-12% difference between the ED plateaux obtained for three samples that were each processed twice, by different operators. A random uncertainty of 20% is adopted for the Malangangerr samples to accommodate their poorer growth curve fits. The standard error of the plateau mean is typically around 1%. Systematic uncertainties are estimated at 3%.

|| Mean ± (random uncertainty, total uncertainty). The mean age is calculated after subtracting a surface residual ED of 1 ± 1 Gy. The random uncertainty should be used to compare TL dates at a site and the total uncertainty used to compare TL dates between sites or with radiocarbon dates.

that the lowest artefacts may have been trodden into unconsolidated older sediments by the first occupants, a conservative estimate places initial occupation at 2.4 m depth. KTL162 and 164 were collected from around 2.6 and 2.3 m depth respectively and bracket the date of first occupation to between 61 ± 13 and 45 ± 9 kyr. A confirmatory date of 52 ± 11 kyr is provided by the intervening sample (KTL158). Further confidence in KTL164 as a secure *terminus ante quem* for human occupation is given by the presence of artefacts in a small pit overlain by the sediments from which this TL sample was taken.

TL dating has the potential to provide reliable dates for sediments in many depositional environments⁵⁻⁷. We obtained TL dates from quartz grains of 80-115 μm diameter using the regenerative ($N + UV + \beta$) and additive-dose ($N + \beta - I_0$) methods^{8,9}. N and I_0 are the respective natural and residual TL levels of a sample, whereas UV and β indicate the use of laboratory bleaching and irradiation procedures respectively. The residual TL level¹⁰ was taken as that attained after a 20 h sunlamp bleach as the sand apron deposits are well bleached before burial. Four lines of evidence support this assertion. First, the grains bleach to a low level within 2 h of exposure to the sunlamp or natural sunlight (Fig. 2). Second, sand grains receive high insolation before burial: using an escarpment retreat rate of 1 m kyr⁻¹ (ref. 11), a grain is exposed on the escarpment face for many months and, once eroded, it may then remain exposed for several years on the apron surface. Third, sand grains sampled from the ground surface of two other sand aprons in the same catchment have low natural TL levels, with a mean equivalent dose (ED) of 0.9 ± 0.1 Grays (Gy). Fourth, the ED of samples collected from 1 cm beneath the ground surface at the two sites is only marginally greater: KTL156 and 157 have an ED of 1.2 ± 0.2 Gy and 1.5 ± 0.3 Gy respectively (Table 1).

Linear and saturating exponential least-squares fits were attempted on the first-glow growth curves. Saturating exponential curves gave close fits for both near-surface (KTL156 and 157) and buried samples when dosed to 300 Gy; the quartz begins to saturate above 200 Gy. For the buried samples, the additive-dose points were superimposed on to their corresponding regenerated growth curves (Fig. 3): the former lay invariably within the scatter about the latter. This lack of sensitivity change

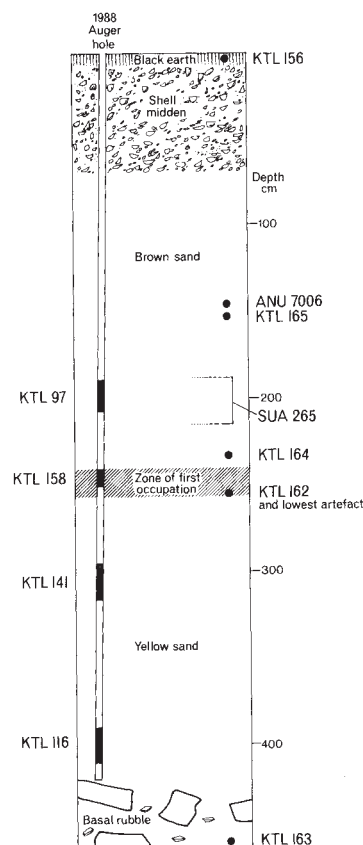


FIG. 1 Stratigraphic section of the Malakunanja II sand apron excavation (SW face). The location and depth interval of TL and ^{14}C samples is shown for the auger hole (left) and excavation pit (right). Radiocarbon sample SUA-265 was collected previously³ from deposits separated laterally from our excavation by about 1 m.

suggests that the regenerative method is suitable for ED determination. Regenerative growth curve intercepts were calculated at 5 °C intervals between 200 and 500 °C and an ED plateau was sought in the region above 260 °C (Table 1; Fig. 3). Samples processed under yellow filters (which transmit light >470 nm) had two plateau regions: at 260–320 °C and at 330–500 °C. The lower plateau may have resulted from a partial reduction of the 325 °C peak. Only the upper plateau is stratigraphically consistent with the single plateau obtained from samples processed under primary red filters (which transmit light >580 nm); these include the key samples KTL162 and 164. For 'double' plateau samples, the upper region supplied the ED used in the age calculation. The near-surface samples yielded ED plateaux with both the regenerative and additive-dose methods: the mean value was adopted for the age calculation and the random uncertainty incorporated the spread between the pair. The uncertainty about the mean plateau value was estimated at 10% for the other Malakunanja II samples and at 20% for those from Malangangerr. An approximate zero-age correction was applied to the ED of each sample by deducting a surface residual of 1 ± 1 Gy; this value is small in comparison with the ED of the buried samples, being of the same order as the estimate of uncertainty.

The environmental dose rate was deduced from radionuclide concentrations measured by high-resolution γ -ray spectro-

metry¹². Sediment samples were collected from within the 30 cm radius around each TL sample and their radionuclide activities determined (Table 1). The results are consistent with a condition of secular equilibrium for each sample and down both profiles. As nuclide migration is not apparent at either site, secular equilibrium is assumed to have prevailed since sample deposition. From the measured activities, β and γ dose rates were calculated¹³ and a correction made for the sand apron moisture content. Smaller contributions to the total dose are derived from cosmic rays^{14,15} (10–20%) and the internal α activity of etched quartz (~2%).

The TL ages are considered to be reliable for two reasons. First, all dates are in correct stratigraphic order with a modern age for the near-surface samples. This is consistent with the continuous development of sand aprons at both sites. There is no field evidence, such as a marked break in depositional sequence or the hydraulic sorting of sediments, to suggest that either site has been fluvially disturbed. Second, the ¹⁴C dates and our corresponding TL dates are in close accordance. The TL samples of most archaeological importance are KTL158, 162 and 164. The latter pair bracket the first human use of Malakunanja II and suggest that people first arrived at this site between 61 ± 13 and 45 ± 9 kyr. This claim is supported by the date of 52 ± 11 kyr obtained from the interposed sample (KTL158). Humans arriving in Australia from the Indo-Malay-

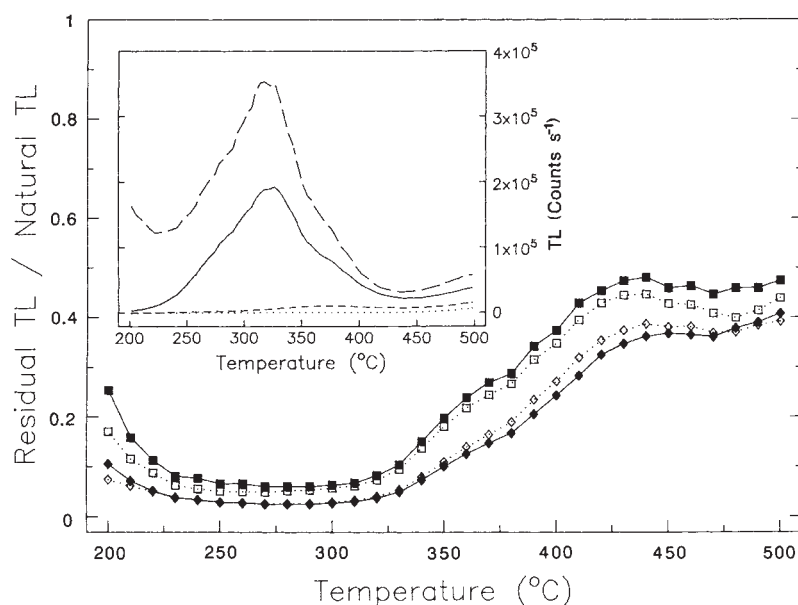
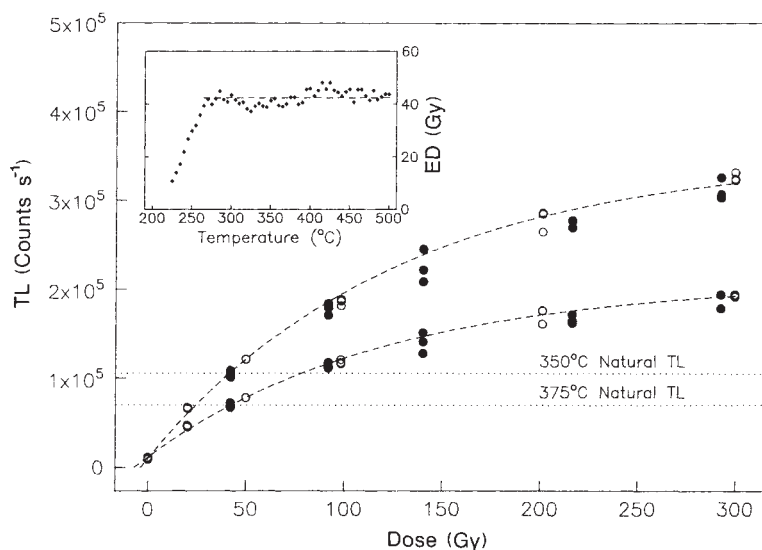


FIG. 3 Regenerative growth curves (---) at 350 °C (upper curve) and 375 °C (lower curve) and ED plateau (inset) for KTL164. ED intercepts are shown at 5 °C intervals and the mean plateau value is marked by the dashed line. Main figure: ○, regenerative dose point; ●, additive dose point. Each dosage level has three replicates.

FIG. 2 TL glow curves (inset) and bleaching curves for KTL164. Samples were heated in a nitrogen atmosphere to 500 °C at a rate of 2.5 °C s^{-1} . The TL signal was detected with an EMI 9635B photomultiplier tube through Corning 7-59 and Chance-Pilkington HA-3 filters. Laboratory irradiations were conducted with a ⁹⁰Sr/⁹⁰Y β source delivering 5 Gy min^{-1} and an unfiltered Philips MLU 300 W sunlamp was used for bleaching. Sample replicates were normalized by weight to $8.0 \pm 0.2 \text{ mg}$. Anomalous fading¹⁶ was not detected in selected samples stored for up to 58 weeks. Inset figure: —, natural TL; —, $N + 50 \text{ Gy}$; ---, $N + 20 \text{ h UV}$; ····, incandescence. Main figure: ■, 2 h UV; ◆, 20 h UV; □, 2 h SUN; ◇, 20 h SUN.



sian region would have encountered the prominent Arnhem Land escarpment on moving inland. Malakunanja II occupies a strategic location along its base and may have been brought into use within a few thousand years of first landfall. Any delay is probably accommodated within the estimates of uncertainty about the TL dates. The absence of artefacts in deposits older than 60 kyr at this key site suggests an upper limit on the time of arrival of modern humans in Australia. □

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Iron in Antarctic waters

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WE are testing the hypothesis that Antarctic phytoplankton suffer from iron deficiency^{1–3} which prevents them from blooming and using up the luxuriant supplies of major nutrients found in vast areas of the southern ocean. Here we report that highly productive⁴ ($\sim 3 \text{ g C m}^{-2} \text{ day}^{-1}$), neritic Gerlache Strait waters have an abundance of Fe (7.4 nmol kg^{-1}) which facilitates phytoplankton blooming and major nutrient removal, while in low-productivity⁴ ($\sim 0.1 \text{ g C m}^{-2} \text{ day}^{-1}$), offshore Drake Passage waters, the dissolved Fe levels are so low ($0.16 \text{ nmol kg}^{-1}$) that the phytoplankton are able to use less than 10% of the major nutrients available to them. The verification of present-day Fe deficiency is of interest as iron-stimulated phytoplankton growth may have contributed to the drawing down of atmospheric CO_2 during glacial maxima^{2,3}; it is also important because oceanic iron fertilization aimed at the enhancement of phytoplankton production may turn out to be the most feasible method of stimulating the active removal of greenhouse gas CO_2 from the atmosphere, if the need arises (J.H.M., manuscript in preparation).

Seawater samples were collected using Teflon-coated, 30-litre Go-Flo bottles suspended on Kevlar line. After filtering through $0.4 \mu\text{m}$ Nucleopore filters, metals were pre-concentrated from the water by organic extraction⁵ or, in the case of Mn, Chelex-100 ion exchange⁶. Small chunks of floating ice were gathered and placed in acid-cleaned plastic bags. After allowing the outer surfaces to melt away, the remaining ice was rinsed with Milli-Q water; meltwater was then collected in bottles and acidified with 4 ml of 6M HCl l^{-1} . As the ice samples were not filtered, the values reported here represent the metals dissolved in the melted water plus the metals that dissolved from particulates when the

acid was added and pH lowered to ≈ 1.9 – 2.0 . Analyses were performed by graphite furnace atomic absorption spectrophotometry. All procedures were carried out using stringent anti-contamination techniques⁷.

In the offshore waters of the Drake Passage (Station 2), Fe concentrations in 10 samples ranged from $0.16 \text{ nmol kg}^{-1}$ near the surface to $1.55 \text{ nmol kg}^{-1}$ at $1,850 \text{ m}$ (Table 1). However, when the Fe data were plotted against depth, a 'saw-toothed' profile was observed. It was subsequently determined that in spite of rigorous cleaning, all samples taken from Go-Flo bottle No. 1 had an extra half nmol or so of Fe as well as excess Mn and Co. Nevertheless, the five remaining 'clean' Drake Passage samples are sufficient for initial comparisons of concentrations with those from other offshore waters with an excess of major nutrients, such as the Gulf of Alaska, as well as comparisons with Antarctic neritic waters, such as those from the Gerlache Strait.

Very similar Fe depth distributions were observed in the Drake Passage and the Gulf of Alaska (Ocean Station 'PAPA'; 50°N ; 145°W), that is, very low (≈ 0.10 – $0.16 \text{ nmol kg}^{-1}$) surface values and increasing concentrations with depth (Fig. 1). As was the case with the Alaska data, increasing Fe values in Drake Passage more or less go along with increasing major nutrient concentrations and decreasing oxygen levels (Table 1); there are not enough data for meaningful regression analysis, however.

Surface Co concentrations ($\approx 25 \text{ pmol kg}^{-1}$) were about the same in both regions; however, the subsurface maximum found in Alaska was not observed here. The Mn maximum commonly found in association with northeast Pacific oxygen minima was also missing (Fig. 1), as would be expected, as subsurface Drake Passage oxygen concentrations do not fall below $180 \mu\text{mol kg}^{-1}$. The surface Mn concentration, $0.08 \text{ nmol kg}^{-1}$, is markedly lower than that observed at 'PAPA' ($0.52 \text{ nmol kg}^{-1}$). Depth profiles of other elements such as Cu, Cd and Zn (Table 1) were similar to those observed in the northeast Pacific.

Neritic Gerlache Strait concentrations of Fe and Mn were 50–60 times higher than those measured in the open-ocean waters of the Drake Passage (Table 1, Fig. 2). Assuming that the Fe requirement of phytoplankton in relation to nitrogen is about 5000N:1Fe (ref. 2), the $7.4 \text{ nmol Fe kg}^{-1}$ is more than enough to support the removal of the $\approx 24 \mu\text{mol NO}_3 \text{ kg}^{-1}$ occurring in the Gerlache. Clearly, there is no Fe limitation, a fact that we believe helps to explain the very high productivity rates reported here ($3 \text{ g C m}^{-2} \text{ day}^{-1}$) (ref. 4) and in other shallow southern ocean coastal regions that should be equally rich in Fe (for example, South Georgia⁸ and Deception Island⁴).

In contrast to Fe-rich, shallow, inshore waters, it is apparent that very little Fe is available in offshore Drake Passage waters. For example, if we use an upwelling rate of 0.25 m day^{-1} (ref. 9), the NO_3 upwelling to the surface each day ($25 \text{ mmol NO}_3 \text{ m}^{-3} \times 0.25 \text{ m day}^{-1} = 6.25 \text{ mmol m}^{-2} \text{ day}^{-1}$) would be enough to support rates of new phytoplankton productivity of the order of $40 \text{ mmol C m}^{-2} \text{ day}^{-1}$ ($6.25 \text{ mmol NO}_3 \times \text{C:N Redfield ratio of } 6.6$). With the same upwelling rate, and assuming a phytoplankton C:Fe demand ratio of 33,000:1 (ref. 2), the $\approx 0.10 \mu\text{mol Fe m}^{-3}$ upwelling with the NO_3 would support only about $1 \text{ mmol C m}^{-2} \text{ day}^{-1}$. Even using the highest C:Fe ratio¹⁰ for iron-starved phytoplankton (100,000:1) would result in little more than $3 \text{ mmol C m}^{-2} \text{ day}^{-1}$; that is, less than 10% of the carbon that could be fixed with the upwelled nitrogen.

Clearly, insufficient Fe is introduced by upwelling; and as this station is well offshore (230 km from the Antarctic Peninsula), the Fe necessary for phytoplankton growth would have to come from an alternative source such as fallout of atmospheric dust. But, this region is known for having the lowest atmospheric dust loads in the world: Prospero¹¹ reports only 0.5 ng Fe for the South Pole in comparison to 9 and 90 ng Fe m^{-3} for offshore islands such as Oahu and Bermuda.

The low rates of atmospheric dust input are exemplified by the surface Mn concentration observed at this station,

TABLE 1 Hydrographic, nutrient and trace metal data from the March/April 1989 *Polar Duke* cruise

Depth (m)	Potential temperature (°C)	Salinity (‰)	σ_t (g l ⁻¹)	O ₂	NO ₃	PO ₄	SiO ₃	Fe	Mn	Co	Cu	Cd	Zn
Station 1, 56°38'S; 65°20'W, 27 March 1989, North Drake Passage													
50	6.54	34.035	26.72	274	23.0	1.60	2.0	0.16	0.26	27.1	0.71	0.26	0.24
Station 2, 60°46'S; 63°26'W, 28 March 1989, South Drake Passage													
30	2.97	33.798	26.93	314	24.8	1.78	8.8	0.16	0.08	25.4	0.97	0.28	0.63
60	2.96	33.802	26.93	315	25.5	1.76	12.3	(0.52)	(0.20)	(43.3)	1.05	0.28	0.30
110	-0.34	33.928	27.26	341	29.3	2.12	25.7	0.10	0.21	25.8	1.12	0.56	1.49
200	1.51	34.250	27.41	300*	33.9	2.39	51.7	(0.85)	(0.45)	(56.3)	1.46	0.75	3.86
300	1.89	34.399	27.50	225*	35.1	2.54	64.7	0.26	0.25	28.6	1.52	0.81	4.74
400	2.05	34.491	27.56	200*	35.5	2.56	70.2	(0.88)	(0.48)	(47.8)	1.71	0.80	5.10
550	2.11	34.588	27.63	180*	35.4	2.55	89.8	0.40	0.29	27.4	1.68	0.77	5.25
1000	1.92	34.703	27.74	180*	33.3	2.42	96.0	(0.88)	(0.49)	(40.1)	1.90	0.70	5.46
1420	1.56	34.723	27.78	185*	30.3	2.28	101.0	0.76	0.31	21.1	2.07	0.66	5.67
1850	0.80*	34.713*	27.86*	205*	31.6	2.40	108.0	(1.55)	(0.77)	(63.8)	2.38	0.66	6.34
Station 3, 64°55'S; 63°19'W, 1 April 1989, Gerlache Strait													
15	0.92	33.641	26.95	317	23.8	2.19	74.3	7.40	5.05	82.0	2.24	0.56	4.90
50	1.05	33.865	27.13	310	22.9	2.29	81.5	4.70	4.19	58.7	2.09	0.60	5.10
200	0.31	34.389	27.60	272	30.7	2.65	94.0	6.85	3.86	82.0	2.16	0.70	5.90
ice	—	—	—	—	—	—	—	25.9†	—	56.6	0.02	0.02	—

Values for O₂, NO₃, PO₄, SiO₃ are given in $\mu\text{mol kg}^{-1}$, Fe, Mn, Cu, Cd, Zn in nmol kg^{-1} , Co in pmol kg^{-1} .

* Data estimated from GEOSECS Station 78, 61°3'S; 62°58'W, 3 January 1973 (ref. 20). † Total dissolvable Fe. () Contaminated; all taken from Go-Flo No. 1. σ_t is the density of sea water in g l^{-1} based on salinity and potential temperature.

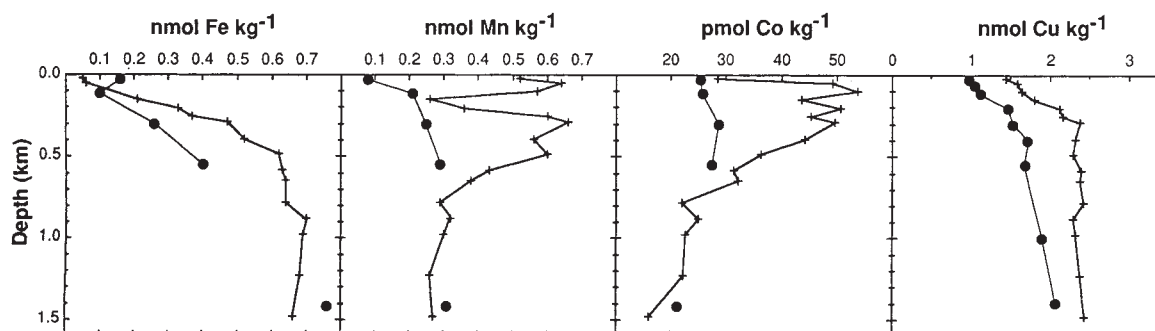


FIG. 1 Drake Passage (Station 2) dissolved Fe, Mn, Co and Cu concentrations

(●) compared with those from the Gulf of Alaska (+) Station 'PAPA' (ref. 2).

0.08 nmol kg^{-1} . In their comprehensive study of Mn in Pacific Ocean surface waters, Klinkhammer and Bender¹² noted that the correlation between Mn and ²¹⁰Pb suggested aeolian control of surface ocean Mn. They also reported that Mn concentrations were lowest in the Southern hemisphere: 0.3 nmol kg^{-1} , a value that is about four times higher than the 0.08 nmol kg^{-1} we measured in the Drake Passage. To our knowledge, this Mn concentration is the lowest yet reported for surface water in the world ocean; it suggests that Mn deficiency may also be a factor contributing to the limitation of phytoplankton growth in the southern ocean.

In addition to the input from Fe-rich sediments in shallow waters and atmospheric dust in offshore waters, a third Fe supply mechanism exists in the southern ocean—Fe released from melting sea ice. On the basis of the amounts of Fe measured in shallow snow pits (0.02 nmol Fe g^{-1}) (ref. 13) and Holocene ice cores (0.03 nmol Fe g^{-1}) (ref. 14), Martin³ estimated that the phytoplankton could fix about 150 mmol C m^{-2} with the Fe made available as sea ice melts during the austral spring and summer (estimates based on 10% available Fe and a C:Fe ratio of 33,000:1). We report here an initial ice Fe concentration of 0.026 nmol g^{-1} (Table 1), an amount that agrees well with the values mentioned above. This was glacial ice of unknown age, however, not annual sea ice. Determining the Fe content of annual sea ice is important in view of the amounts of ice involved: Munk¹⁵, for example estimates that 2.1×10^{19} g of ice forms and melts back each year. Obviously, the biological availability as well as the Fe content of the annual ice will need to

be known if accurate estimates are to be made of new phytoplankton productivity that could be supported by this potentially important Fe input mechanism.

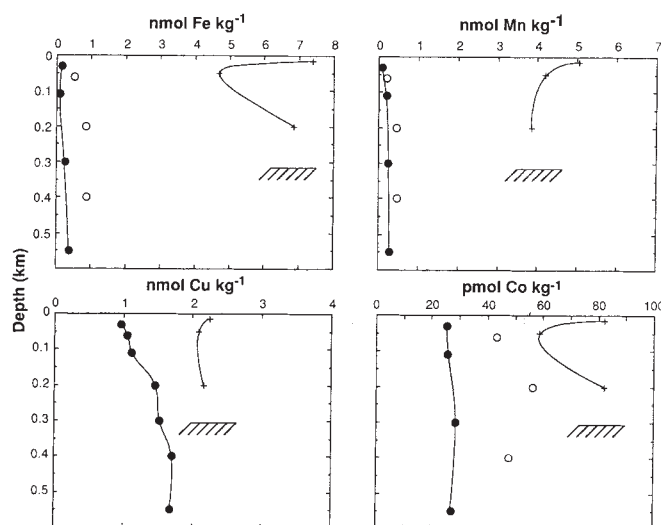


FIG. 2 Drake Passage Fe, Mn, Co and Cu concentrations (●) compared with those (+) from the Gerlache Strait (Station 3). Open circles are contaminated samples collected via Go-Flo No. 1.

In conclusion, the results presented here clearly support the observations of the 1925–27 *Discovery* Expedition scientists—that the neritic areas probably were (and are) rich in phytoplankton because of Fe introduced from land^{8,16,17}. The very low dissolved Fe (and Mn) levels in the Drake Passage provide support for our argument^{2,3} that present-day plant productivity is limited in offshore waters of the Antarctic because of Fe deficiency. Judging by the unused excess of major plant nutrients, this lack of essential Fe (ref. 18) seems to be severely limiting the power of the 'biological pump' and thus contributing to the raised atmospheric CO₂ concentrations typical of previous and present interglacial periods (preindustrial ≈280 p.p.m.) (ref. 19). In contrast, greatly enhanced Fe input from atmospheric dust^{2,3,14} may have stimulated phytoplankton growth and increased the power and efficiency of the biological pump, thus contributing to the drawing down of atmospheric CO₂ during glacial maxima¹⁹.

Note added in proof. We have since shown in experiments in the Ross Sea that Fe will indeed stimulate plankton productivity in the austral spring and summer in amounts that may be relevant to the removal of excess CO₂ from the atmosphere. Further details will be published elsewhere. □

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Glucagon stimulates the cardiac Ca²⁺ current by activation of adenylyl cyclase and inhibition of phosphodiesterase

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GLUCAGON exerts positive inotropic and chronotropic effects in the heart^{1,2}. Like its glycogenolytic effect in liver cells³, the cardiac effects of glucagon are often correlated with adenylyl cyclase stimulation^{4–7}. Therefore, cyclic AMP-dependent phosphorylation of L-type Ca²⁺ channels^{8–10} might be involved in the inotropic effect of glucagon. There have been no reports, however, of the effects of glucagon on the cardiac Ca²⁺ current (*I*_{Ca}). Also, the physiological effects of glucagon could involve mechanisms other than stimulation of adenylyl cyclase^{11,12}. Here we show that glucagon enhances *I*_{Ca} in frog and rat ventricular myocytes. The effect of glucagon in rats resulted from a stimulation of adenylyl cyclase. In frogs, however, the effect of glucagon on *I*_{Ca} was smaller and occurred at a concentration tenfold lower than in rats, and adenylyl cyclase was not modified. In addition, cAMP potentiated the effect of glucagon on *I*_{Ca} in frog ventricle, which correlated with the observed inhibition by glucagon of low-*K*_m cAMP phosphodiesterase activity. Therefore, this is an example of a hormone that affects cardiac function in a similar way to a variety of synthetic cardiotonic compounds, such as milrinone and Ro-20-1724 (ref. 13). Inhibition of phosphodiesterase activity by glucagon may be essential in animals in which glucagon increases cardiac contractility but does not effectively stimulate adenylyl cyclase^{7,14,15}.

The effects of 1 and 10 μM glucagon on *I*_{Ca} were recorded from a frog (Fig. 1a) and rat (Fig. 1b) ventricular cell using the whole-cell patch-clamp technique. Glucagon increased *I*_{Ca} in both preparations in a dose-dependent and reversible way. Significant differences were found, however, between the two species. In frog, glucagon increased *I*_{Ca} with a 50% stimulatory

concentration (EC₅₀) of 41 nM (Fig. 1c), whereas in rat, the EC₅₀ was more than 10 times this value and the maximal stimulation of *I*_{Ca} was more than three times that in frog (Fig. 1d). Furthermore, not all the frog cells responded to glucagon, whereas every rat ventricular cell did. The lack of responsiveness to glucagon in some of the frog cells was probably due to a notably low level of cAMP (see below).

The effects of glucagon in rat quantitatively resembled the effects of isoprenaline, a β-adrenergic agonist. Furthermore, maximal concentrations of isoprenaline and glucagon did not have an additive effect on *I*_{Ca} (data not shown), and the effects of glucagon on *I*_{Ca}, like those of isoprenaline, were strongly antagonized by acetylcholine (ACh) (Fig. 2a). This indicates that glucagon and isoprenaline act through similar mechanisms in the rat. As β-adrenergic stimulation of *I*_{Ca} is essentially due to activation of adenylyl cyclase^{8–10}, we investigated the effects of glucagon on the activity of this enzyme in rat ventricle. As reported previously^{2,4–7}, glucagon increased adenylyl cyclase activity in a dose-dependent way (Fig. 2b), and its effects were antagonized by ACh¹⁶.

In frog, the stimulatory effect of glucagon on *I*_{Ca} was not reduced by ACh (Fig. 2c), in contrast to the activation of *I*_{Ca} by isoprenaline¹⁰. Moreover, maximal activation of *I*_{Ca} by glucagon was about 15 times smaller than that evoked by isoprenaline (Fig. 2c). Finally, whereas isoprenaline enhanced adenylyl cyclase activity in a dose-dependent way in frog ventricle, glucagon was unable to modify either basal or isoprenaline-stimulated adenylyl cyclase activity in this preparation (Fig. 2d). Therefore, we concluded that the stimulatory effect of glucagon on *I*_{Ca} in frog cardiac cells was not mediated by enhanced cAMP production.

Cyclic AMP, however, did seem to be involved in *I*_{Ca} stimulation by glucagon in frog cells. This was suggested by a comparison of the effect of a first application of glucagon to a cell under basal conditions with a subsequent application of the peptide after *I*_{Ca} had been stimulated by isoprenaline or cAMP. In nine cells in which the basal *I*_{Ca} was 169.7 ± 22.2 pA (mean ± s.e.m.), an initial application of 3 μM glucagon for 5–6 min increased *I*_{Ca} by only 7.6 ± 7.2% on average, because five of these nine cells did not respond to the peptide (Fig. 3). After the peptide was washed out, application of submaximal concentrations of isoprenaline (0.1 or 1 μM) or perfusion of the patch pipette^{17,18} with cAMP (1 or 3 μM) enhanced *I*_{Ca} to 969.2 ± 237.1 pA. A subsequent application of glucagon further increased *I*_{Ca} by 36.4 ± 8.8% (Fig. 3). Also, glucagon increased *I*_{Ca} more consistently in frog cells under stimulated conditions

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as compared with basal conditions, as 24 out of 27 cells responded to $3 \mu\text{M}$ peptide when applied after elevation of I_{Ca} by isoprenaline or cAMP. Therefore, the stimulatory effect of glucagon on I_{Ca} in frog cardiac cells was facilitated by an increase in intracellular cAMP.

In this respect, the action of glucagon on I_{Ca} resembles the stimulatory effect on I_{Ca} of phosphodiesterase (PDE) inhibitors^{19,20}. We therefore investigated the effects of glucagon on PDE activity in frog ventricles. At least four different types of PDEs have been described in cardiac tissue with different affinities for substrate cAMP¹³. Ro-20-1724 and milrinone are specific inhibitors of type IVa and b, respectively, low- K_m cAMP

PDEs in cardiac preparation¹³. They strongly reduced frog cardiac PDE activity when cAMP concentration was low (Fig. 4a). It is of interest that PDE activity was reduced by glucagon ($1 \mu\text{M}$) in the same low range of cAMP concentrations (0.1 – $2 \mu\text{M}$; Fig. 4a). Glucagon inhibited PDE activity in a dose-dependent way (Fig. 4b), with a 50% inhibitory concentration (IC_{50}) of 26 nM and a maximal inhibition of about 40%. In addition, PDE activity in the cytosolic fraction was poorly sensitive to milrinone and was not inhibited by glucagon (data not shown). In rat ventricle, glucagon had no significant effect on PDE (Fig. 4c), although a low- K_m cAMP PDE activity must have also been present in this preparation, given the inhibitory

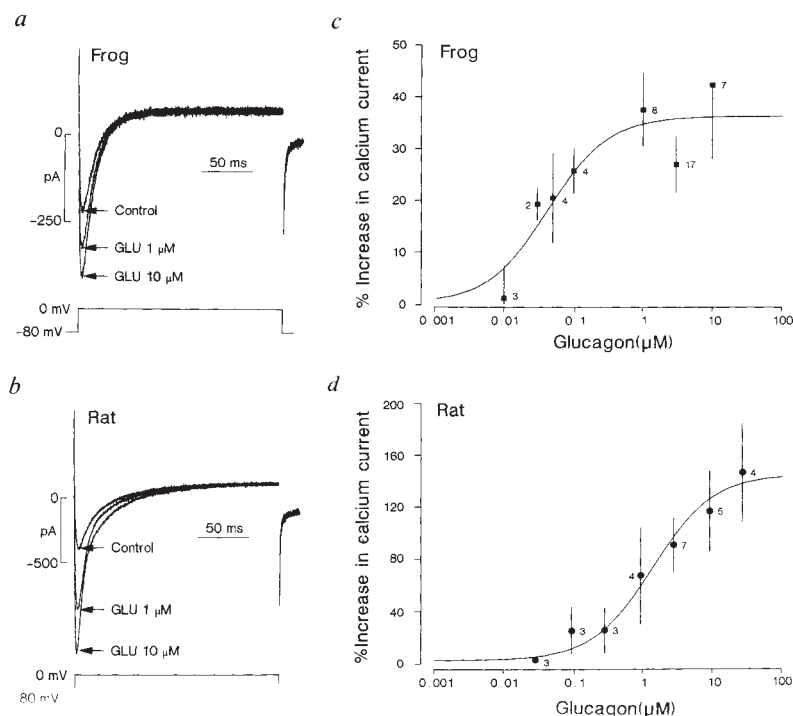
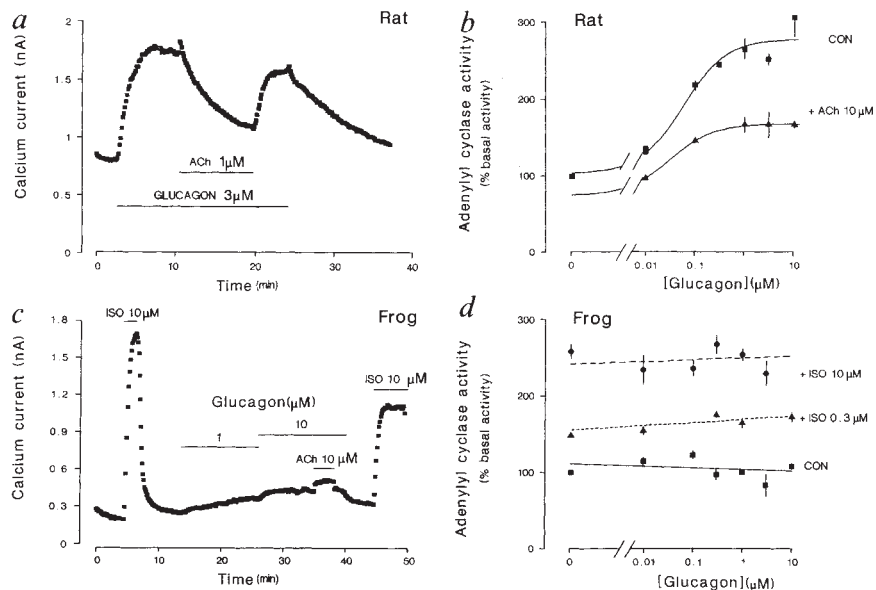


FIG. 1 Effect of increasing concentrations of glucagon on I_{Ca} , recorded with the whole-cell patch-clamp technique^{10,17,18} from frog (a, c) and rat (b, d) ventricular cells. a, b, Individual current traces from a frog (a) and a rat (b) ventricular cell, in control external solutions, and after 5–6-min application of 1 and $10 \mu\text{M}$ glucagon (GLU). c, d, Concentration–response curves of glucagon on I_{Ca} in frog (c) and rat (d) ventricular cells. The points show the mean $\pm$ s.e.m. of the number of cells indicated near the symbols. The data were fitted to the Michaelis equation.

METHODS. Ventricular cells were enzymatically dispersed from frog (*Rana esculenta*) or rat (male Wistar, 200–250 g) as described previously^{18,24}. Control external solution contained (mM): 88.4 (frog) or 108.4 (rat) NaCl; 20 CsCl; 23.8 NaHCO_3 ; 0.6 NaH_2PO_4 ; 1.8 MgCl_2 ; 1.8 CaCl_2 ; 5 D-glucose; 5 sodium pyruvate; 3×10^{-4} (frog) or 5×10^{-2} (rat) tetrodotoxin; pH 7.4, maintained with 95% O_2 , 5% CO_2 . Patch electrodes (0.8 – $1.6 \text{ M}\Omega$) were filled with standard internal solution which contained (mM): 119.8 (frog) or 139.8 (rat) CsCl; 5 K_2EGTA ; 4 MgCl_2 ; 5 Na_2CP ; 3.1 Na_2ATP ; 0.42 Na_2GTP ; 0.062 CaCl_2 (pCa 8.5); 10 HEPES buffer; pH 7.1 adjusted with KOH. In each experiment, the cell was depolarized every 8 s from -80 mV to 0 mV for 200 ms and I_{Ca} was measured as described previously^{10,17,18}. Frog glucagon is very similar to porcine glucagon, with the single Thr 29 substituted by the homologous amino acid, Ser (ref. 25); this modification may not account for physiological response differences. All electrophysiological experiments were performed at room temperature (21 – 24°C). Crystallized porcine glucagon, Novo (Denmark); isoprenaline, acetylcholine and cAMP, Sigma (USA).

FIG. 2 Does adenylyl cyclase participate in the stimulatory effects of glucagon on I_{Ca} ? a, Time course of the changes of I_{Ca} in a rat ventricular cell. The cell was initially exposed to the control external solution and, during the periods indicated, to glucagon ($3 \mu\text{M}$) or to ACh ($1 \mu\text{M}$) in the presence of glucagon. b, Concentration–response curve of the effects of glucagon on adenylyl cyclase activity measured in a membrane fraction of rat ventricle, in the absence (■) or presence of $10 \mu\text{M}$ ACh (▲). c, Time course of the changes of I_{Ca} in a frog ventricular cell. The cell was initially exposed to the control external solution and, during the periods indicated, to isoprenaline (ISO, $10 \mu\text{M}$), glucagon (1 and $10 \mu\text{M}$) or to ACh ($10 \mu\text{M}$) in the presence of glucagon. d, Concentration–response curve of glucagon on adenylyl cyclase activity measured in a membrane fraction of frog ventricle, in the absence (■) or presence of $0.3 \mu\text{M}$ ACh (▲) or $10 \mu\text{M}$ ISO (●).

METHODS. Membranes were prepared as described²². Protein was determined using Bio-Rad assay (Bio-Rad, FRG). Adenylyl cyclase activity was measured as described²⁶. The assay medium contained, in a final volume of $60 \mu\text{l}$: 3 mM [α - ^{32}P]ATP ($3 \times 10^6 \text{ c.p.m.}$); 5 mM MgCl_2 ; $50 \mu\text{M}$ Na_2GTP ; 1 mM EDTA; 1 mM cyclic [8 - ^3H]AMP ($15,000 \text{ c.p.m.}$); 0.2 mM methylisobutylxanthine; 25 mM HEPES buffer; pH 7.6; 25 mM Na_2CP and 1 mg ml^{-1} creatine phosphokinase, and 40 – $60 \mu\text{g}$ membrane protein. It was incubated for 20 min at 37°C . The



results, obtained from triplicate determinations, are expressed as a percentage of basal activity (0.22 ± 0.07 , and $0.16 \pm 0.05 \text{ nmol cAMP per mg protein per 20 min at } 37^\circ\text{C}$ in frog and rat, respectively). [α - ^{32}P]ATP (10 – 50 Ci per mmol) and cyclic [8 - ^3H]AMP (20 – 30 Ci per mmol), Amersham.

effects of Ro-20-1724 (Fig. 4c) and milrinone (data not shown).

The work presented here shows that glucagon activates the Ca^{2+} current in cardiac cells by increasing cAMP through two different mechanisms—stimulation of adenylyl cyclase activity and inhibition of PDE activity. These effects were observed in nanomolar and micromolar ranges of glucagon concentrations, which are two or three orders of magnitude higher than the plasma glucagon level, but which correlate with the concentrations at which glucagon exerts its cardiac inotropic effects^{1,2,4-7}.

PDEs have a crucial role in regulating the level of I_{Ca} in cardiac cells¹⁷⁻²⁰. Although a variety of synthetic compounds, such as Ro-20-1724 and milrinone, inhibit type IV low- K_m cAMP PDEs specifically¹³, only stimulatory effects of hormones and neurotransmitters on PDE activity have, until now, been reported, in both cardiac^{21,22} and noncardiac preparations²³. We have now demonstrated the existence of a physiological inhibitor of a low- K_m cAMP PDE activity. Although further experiments are required to identify the type of PDE that is affected by glucagon, and the molecular mechanism used, preliminary

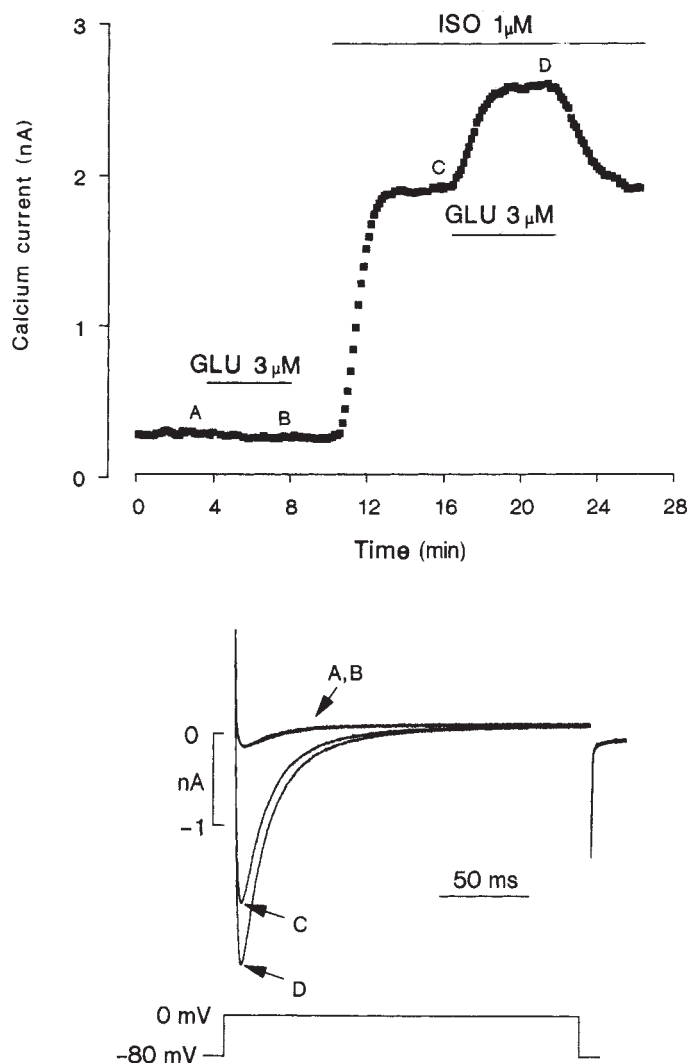


FIG. 3 Isoprenaline facilitates the stimulatory effects of glucagon on I_{Ca} in frog cardiac cells. This is a time course of the changes of I_{Ca} in a frog ventricular cell. The cell was initially exposed to control frog external solution. During the periods indicated, the cell was exposed to glucagon (GLU, 3 μM), isoprenaline (ISO, 1 μM), or to glucagon in the presence of isoprenaline. The current traces shown on the bottom were recorded in control (A), 3 μM glucagon (B), 1 μM isoprenaline (C) and 1 μM isoprenaline with 3 μM glucagon (D) at times indicated on the top graph. Note that in this cell, a stimulatory effect of glucagon on I_{Ca} appeared only after I_{Ca} had been enhanced by isoprenaline.

results indicate that the glucagon-induced inhibition of PDE activity is eliminated in the presence of GDP- β -S. This would indicate that a G protein is involved in the coupling of glucagon receptors and PDE.

The inhibition of PDE activity could participate in the positive inotropic effects of glucagon in animals, such as guinea pig, monkey, and mouse, where the peptide is ineffective in stimulating adenylyl cyclase activity^{7,14,15}. This novel pathway of regula-

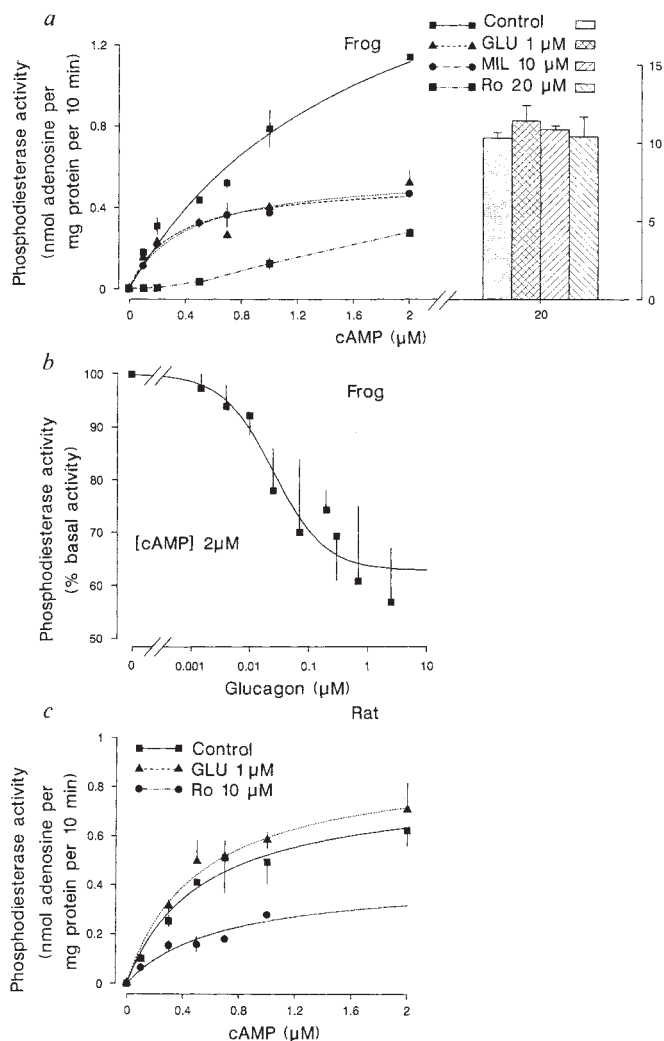


FIG. 4 Effects of glucagon, Ro-20-1724 and milrinone on cAMP PDE activity in frog and rat ventricular cells. *a*, PDE activity was measured in a membrane fraction of frog ventricle as a function of increasing cAMP concentrations in the absence of drugs (control), and in the presence of 1 μM glucagon (GLU), 10 μM milrinone (MIL) or 20 μM Ro-20-1724 (Ro). *b*, Concentration-response curve of glucagon on PDE activity measured in frog ventricular membrane fraction in the presence of 2 μM cAMP. *c*, PDE activity was measured in a membrane fraction of rat ventricle as a function of increasing cAMP concentrations in the absence of drugs, and in the presence of 1 μM glucagon or 10 μM Ro-20-1724.

METHODS. cAMP PDE activity was determined according to the two-step assay procedure described in ref. 27. The assay medium was derived from the frog intracellular solution (see Fig. 1 legend) and contained in a final volume of 0.4 ml: 119.8 mM CsCl; 5 mM K_2EGTA ; 4 mM MgCl_2 ; 62 μM CaCl_2 ; 10 mM HEPES buffer, pH 7.1 adjusted with KOH; 50,000 c.p.m. of [^3H]cAMP; 0.01% bovine serum albumin; 50 μg protein. Incubation, which was initiated by the addition of the proteins, lasted for 10 min at 30 $^\circ\text{C}$ and was terminated by a 45-s ebullition. Data shown in *a* and *c* are the mean $\pm$ s.e.m. of triplicate determinations. Data shown in *b* are the mean $\pm$ s.e.m. of four different experiments. All data points were fitted to the Michaelis equation. Other drugs used in PDE assays were: snake venom (ophiophagus hannah-king cobra, Sigma), milrinone (WIN 47203, Sterling-Winthrop, New York), Ro-20-1724 (Roche).

tion of cell function could also be used for other endogenous substances, and should be explored when investigating the effects of neuropeptides and hormones on cardiac function. □

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Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble *S*-nitrosocysteine than nitric oxide

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STUDIES of cultured bovine aortic endothelial cells using quantitative chemiluminescence techniques have shown that the amount of nitric oxide released under basal conditions, or in response to either bradykinin or the calcium ionophore A23187 is insufficient to account for the vasorelaxant activities of the endothelium-derived relaxing factor (EDRF) derived from the same source¹. This observation contradicts previous suggestions that nitric oxide and EDRF are the same compound², but may be explained if EDRF is a compound that contains nitric oxide within its structure but is a much more potent vasodilator than nitric oxide. Such a molecule could be one of several nitrosothiols which may yield nitric oxide after a one-electron reduction. The present experiments were carried out to test the possibility that the biological activities of the endothelium-derived relaxing factor might more closely

resemble those of one of these compounds, *S*-nitrosocysteine, than nitric oxide. Nitric oxide release from cultured bovine aortic endothelial cells was detected by chemiluminescence and bioassay experiments compared the vasodilator potencies of nitric oxide, *S*-nitrosocysteine, and EDRF. The results suggest that EDRF is much more likely to be a nitrosylated compound such as a nitrosothiol than authentic nitric oxide.

S-nitrosocysteine was synthesized by the reaction of L-cysteine and nitrogen dioxide in cold methanol. Chromatographic separation by HPLC showed nearly 100% conversion of cysteine to *S*-nitrosocysteine by this reaction (Fig. 1).

Nitric oxide in physiological solutions spontaneously degrades to nitrite. The chemiluminescence technique used in this and previous reports^{1,2} requires reflux in a strong reducing environment to convert nitrite formed from nitric oxide back to nitric oxide. This process also cleaves the nitric oxide moiety from a variety of nitroso-compounds³. In the present studies we found that reflux with sodium iodide stoichiometrically cleaved nitric oxide from *S*-nitrosocysteine (Fig. 2a).

The half-lives of *S*-nitrosocysteine, nitric oxide and EDRF were similar, approximately 30 s each (Fig. 2b).

The vasorelaxant activities of nitric oxide, *S*-nitrosocysteine and EDRF were assessed by bioassay. Standard solutions of nitric oxide prepared under anaerobic conditions and maintained in a gas-tight syringe remained virtually pure (Fig. 3). No degradation to nitrite occurred during transit of nitric oxide through the infusion tubing into the bioassay perfusion apparatus (Fig. 3). *S*-nitrosocysteine was found to be approximately 80 times more potent in bioassay than authentic nitric oxide (Fig. 4a). Relaxations in response to either *S*-nitrosocysteine or nitric oxide were consistently reversed by haemoglobin.

The vasorelaxant activities of *S*-nitrosocysteine, nitric oxide and EDRF were compared as a function of the amount of nitric oxide recovered from each compound (measured by chemiluminescence). The relationship between the relaxation produced by basally released EDRF and that released on stimula-

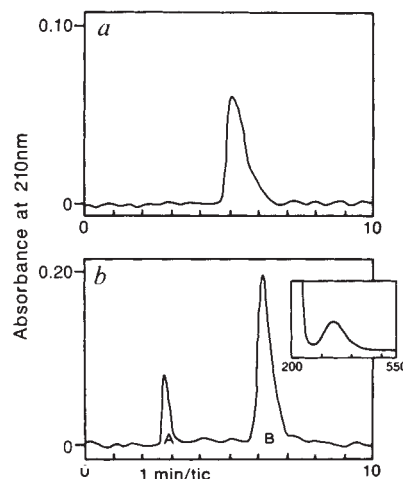


FIG. 1 High-pressure liquid chromatographic separation of *S*-nitrosocysteine from L-cysteine. *S*-nitrosocysteine was synthesized from the reaction of 1 mmol cysteine and 2 mM nitrogen dioxide gas in 1 ml cold methanol. The resulting 1 M *S*-nitrosocysteine solution was stable in methanol at -20°C in the dark. HPLC detection of cysteine and *S*-nitrosocysteine was accomplished using a C-18 reverse phase column with a mobile phase consisting of 20% methanol, 80% 50 mM sodium phosphate, pH 2.2, and 1 mM octane sulphonic acid. Ultraviolet detection at 210 nm was used. a, Cysteine before reaction with nitrogen dioxide; b, after reaction with nitrogen dioxide. Peak A co-migrates with inorganic nitric acid, peak B is *S*-nitrosocysteine. Inset, spectrophotometric analysis of peak B showing absorption characteristic of a nitrosothiol at 340 nm. In methanol or deoxygenated water this compound would not spontaneously yield nitric oxide, as assessed by chemiluminescence.

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tion with the calcium ionophore A23187 and the amount of nitric oxide recovered was quite similar to that observed with *S*-nitrosocysteine, but bore no similarity to the situation when nitric oxide alone was used as a relaxant (Fig. 4b).

One explanation for the difference in potency of EDRF, *S*-nitrosocysteine and nitric oxide relates to the absolute stability of these compounds in bioassay conditions. Although the relative half-lives of these compounds are similar, it is apparent from Fig. 4 that their absolute degradation during a half-life is quite different. The estimated absolute loss of potency of EDRF and *S*-nitrosocysteine in one half-life (30 s) is 0.03 μM , whereas the absolute loss of potency of nitric oxide during a similar period is substantially greater, ranging from 0.5 to 3 μM depending on the precise area of the concentration response curve (Fig. 4) analysed.

Several pharmacological nitrodonors produce their effect by activating guanylate cyclase, a property shared with EDRF (refs 4–6). It has been suggested that nitroglycerine reacts (through unknown intermediates) with thiol groups within vascular smooth muscle to form nitrosothiol compounds^{7,8}. The most abundant source of free sulphhydryl groups in mammalian tissue is cysteine (in either the free or peptide form)⁹. Thus cysteine is a readily available substrate for the formation of nitrosothiols. Although these experiments do not prove conclusively that

EDRF is *S*-nitrosocysteine, the data clearly illustrate that the biological potency of EDRF more closely resembles a nitrosylated compound such as *S*-nitrosocysteine than nitric oxide. We propose that endothelial cells can synthesize and release either *S*-nitrosocysteine or a related nitrosothiol as the EDRF. It is conceivable that EDRF may be composed of several different nitrosothiols, thus accounting for subtle differences in half-life and biological activity observed in different species and

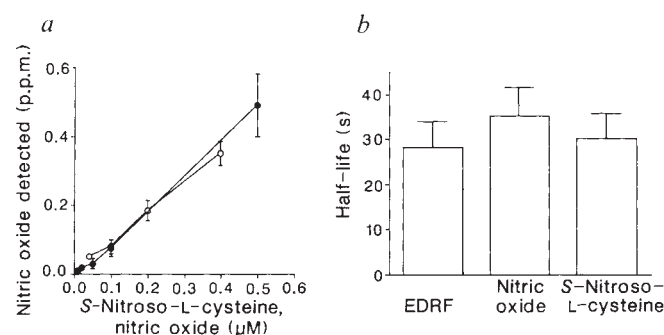


FIG. 2 *a*, Nitric oxide measured by chemiluminescence during infusions of either nitric oxide (○) or *S*-nitrosocysteine (●). Each compound was infused into a reaction chamber containing 1% sodium iodide in glacial acetic acid. Released nitric oxide was transported by a stream of nitrogen gas under vacuum to a chemiluminescence nitric oxide detector similar to that previously described¹. The nitric oxide analyser was initially calibrated using gas of known composition. The instrument was checked for stability daily by infusions of known concentrations of nitric oxide solution. Standard solutions of nitric oxide (water solubility, 7 ml per 100 ml at STP) were prepared daily by dissolving 25 μl of authentic nitric oxide in 25 ml distilled water previously deoxygenated with helium. This gave a stock solution of 4×10^{-5} M. Standard solutions of *S*-nitrosocysteine were prepared in deoxygenated distilled water. Both stock solutions were maintained in gas-tight syringes, and introduced through polyethylene tubing into the bioassay preparation for either measurement of nitric oxide by chemiluminescence or assay of vasodilator activity bioassay. *b*, Half-lives of nitric oxide ($n=6$), *S*-nitrosocysteine ($n=6$), and EDRF ($n=4$) released from cultured bovine aortic endothelial cells in response to the calcium ionophore A23187 (10 μM). Concentrations of nitric oxide and *S*-nitrosocysteine were chosen to produce in excess of 80% relaxation at zero delay. Vasorelaxation was determined using a 3–5 mm segment of porcine left circumflex coronary artery denuded of endothelium and superfused with a physiological buffer containing (in mM) 118.3 NaCl, 4.7 KCl, 2.5 CaCl_2 , 1.2 MgSO_4 , 1.2 KH_2PO_4 , 25 NaHCO_3 , and glucose 11.1. This solution was aerated with 95% O_2 , 5% CO_2 . The detector ring was precontracted with 0.1–1.0 μM prostaglandin $\text{F}_{2\alpha}$. Vasorelaxant activity of each agent was determined using transit times of 3, 12, 20 and 30 s from the site of infusion or site of the endothelial cells to the detector ring. Transit times were varied by a series of stopcocks with varying lengths of interposed tubing immersed in a 37 °C bath. There was a negative linear relationship between amount of vessel relaxation and transit time ($r = -0.97 \pm 0.01$, -0.94 ± 0.03 , and -0.93 ± 0.02 for EDRF, *S*-nitrosocysteine and nitric oxide respectively). Transit time required to produce 50% relaxation relative to the amount observed at zero delay was calculated from the regression equation of each of these relationships.

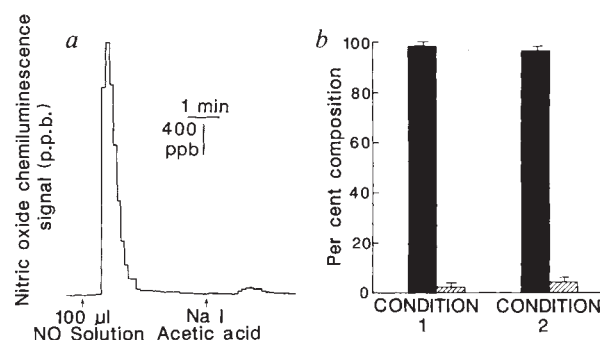


FIG. 3 Relative compositions of nitric oxide and nitrite within standard nitric oxide solutions. Within a specially designed chamber, 20 ml of deoxygenated distilled water was bubbled with nitrogen gas, first passed through sodium dithionite (1 M) to remove trace amounts of oxygen. Aliquots of nitric oxide removed directly from the gas-tight syringe (condition 1, $n=10$) or after passage through polyethylene tubing 30–40 cm in length (condition 2, $n=10$) were injected through a Teflon septum into the chamber, and the purged nitric oxide measured by chemiluminescence. The polyethylene tubing (internal diameter, 0.38 mm, external diameter, 1.09 mm) used for this analysis was identical to that used for the introduction of nitric oxide in the bioassay preparations. The infusion rate for nitric oxide obtained at the end of the perfusion tubing was 0.2 ml min^{-1} , a rate similar to that used for the bioassay experiments. When the nitric oxide signal returned to baseline, excess 1% sodium iodide in glacial acetic acid was infused into the chamber until the remaining nitrite was reduced to nitric oxide, again detected by chemiluminescence. *a*, A sample recording. Areas under the chemiluminescence curves before and after acid reflux were quantified using planimetry. Using this analysis, nitric oxide standard solutions were found to be greater than 95% pure under both conditions 1 and 2(*b*). Solid bars, nitric oxide; hatched bars, nitrite.

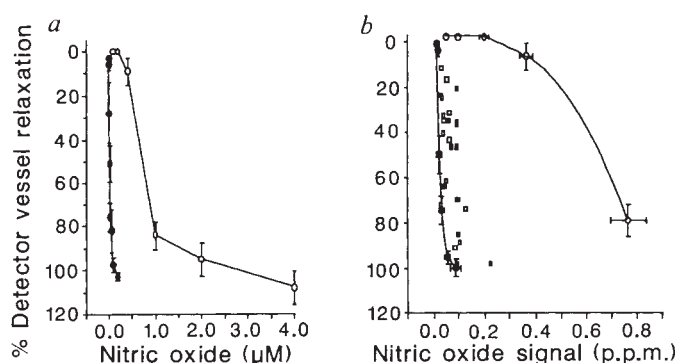


FIG. 4 *a*, Concentration response relationships for vascular relaxation caused by nitric oxide (○) and *S*-nitrosocysteine (●) ($n=4-10$ for each concentration). *b*, Vascular relaxation produced by nitric oxide (○), *S*-nitrosocysteine (●), and EDRF (□, ■) in bioassay, expressed as a function of the amount of nitric oxide detected by chemiluminescence during infusion of identical concentrations of each agent. Both nitric oxide and *S*-nitrosocysteine were prepared as described in the legend to Fig. 2a. All studies were carried out in the presence of indomethacin (1 μM) and after pre-construction of the detector ring with $\text{PGE}_{2\alpha}$ (0.1–1.0 μM). EDRF derived from bovine aortic endothelial cells was released under basal flow conditions (□) and after stimulation with 10 μM calcium ionophore A23187 (■). The transit time from the site of infusion of nitric oxide or *S*-nitrosocysteine was identical to the transit time of EDRF from the cultured endothelial cells (3 s).

vascular beds. In additional studies we examined the potencies and half-lives of two other nitrosothiols, *S*-nitrosoglutathione and *S*-nitrosomercaptoethanol. Both were found to be potent vasodilators with 50% effective doses of approximately 10 nM or less. The half-lives of both compounds, however, were substantially longer than that of EDRF released from bovine aortic endothelial cells, exceeding 2 min ($n = 3$ for both).

The incorporation of nitric oxide into a nitrosothiol compound such as *S*-nitrosocysteine may have several important biological implications. When nitric oxide is incorporated into *S*-nitrosocysteine, its absolute stability and the potency is substantially enhanced. The release of such a compound enables a finer instantaneous control of vascular tone than the release of a less potent compound. The incorporation of nitric oxide into *S*-nitrosocysteine may be important in the control of EDRF secretion. It is conceivable that transmembrane transport relies on the association of nitric oxide with a carrier molecule such as an amino acid or other thiol compounds. Uptake of a nitrosothiol into the vascular smooth muscle may also depend on a transport mechanism. Alternatively, the nitrosothiol may degrade at the smooth muscle cell membrane to yield nitric oxide. This process may more efficiently deliver nitric oxide to the cytoplasm of the vascular smooth muscle.

Several pathological processes are associated with abnormalities of endothelium-dependent vascular relaxation. These include acute hypertension¹⁰, diabetes¹¹, ischaemia with subsequent reperfusion¹², and atherosclerosis¹³. Many of these processes are associated with the generation of oxygen free radicals within endothelial cells which may oxidize free sulphhydryl groups to the disulphide form. This may result in either an

inability of the endothelium to synthesize nitrosothiols, resulting in the release of either nitrite (a compound with essentially no vasodilator activity) or nitric oxide (a compound substantially less potent than EDRF). In either instance, endothelium-dependent vascular relaxation would be impaired by the oxidation of sulphhydryl groups within endothelial cells. □

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Similarity of the product of the *Drosophila* neurogenic gene *big brain* to transmembrane channel proteins

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CELLS in the neurogenic region of *Drosophila* embryos are initially bipotential; they can become either neuroblasts or epidermoblasts^{1,2}. Cell-cell interaction seems to play an important part in this developmental decision³, which involves the function of a group of genes (the neurogenic genes). Loss-of-function mutations in any of the neurogenic genes result in nervous system hyperplasia and epidermal hypoplasia^{4,5}. Of the six known zygotic neurogenic genes, *big brain* (*bib*) is unique in several aspects⁶⁻⁹. Most notably, all the other known neurogenic genes seem to fit into a cascade defined by genetic interactions, whereas *bib* does not show any detectable interaction with them⁹. To understand how *bib* functions, we have now cloned the *bib* genomic and complementary DNAs. The predicted *bib* product shows significant sequence similarity to a family of transmembrane proteins¹⁰⁻¹³, some of which form channels permeable to small molecules^{14,15}. Together with genetic studies, our results indicate that the *bib* product may mediate intercellular communication in a pathway separate from the one involving the products of the other neurogenic genes.

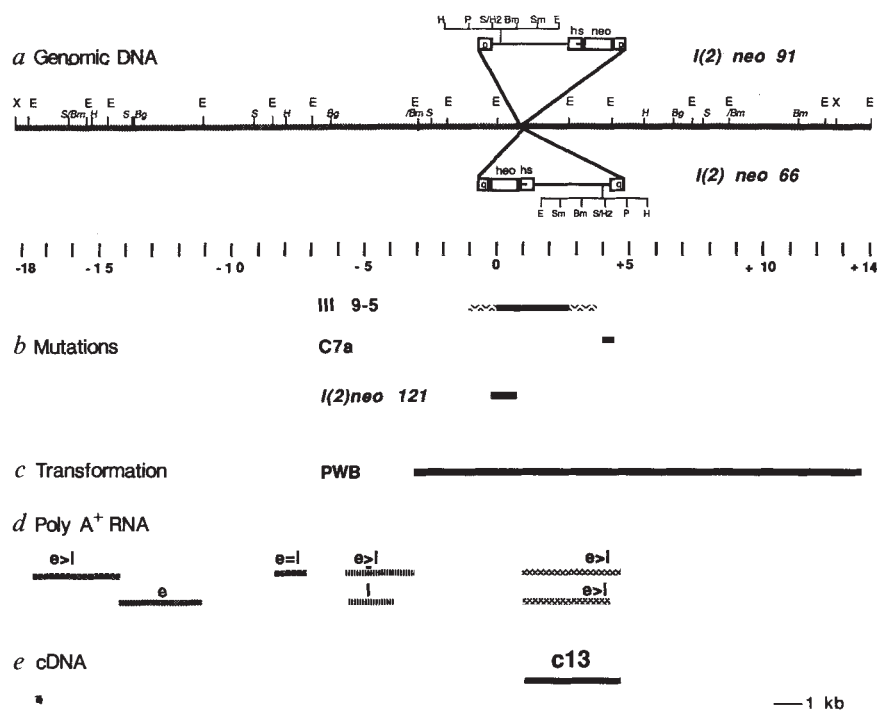
The *bib* locus has been mapped genetically to 30F on the left arm of the second chromosome⁵. By screening embryonic lethal mutations caused by the insertion of a P-element-derived vector¹⁶, we found two neurogenic mutant lines, *l(2)neo66* and *l(2)neo91*, that did not complement *bib* and had the P-element insertion at 30F. To test whether the insertion is the cause of

the *bib* phenotype, we have done a reversion experiment in which the P-element in *l(2)neo91* was excised from its insertion site after exposure to transposase activity carried on a different chromosome. The neurogenic phenotype and lethality reverted with the removal of the P-element, demonstrating that the insertion at 30F in this case was indeed responsible for the phenotype.

Genomic DNA surrounding the P-element insertion sites in *l(2)neo66* and *l(2)neo91* has been cloned by plasmid rescue¹⁷ (Fig. 1a). When northern blots of embryonic RNA were hybridized with these genomic DNA fragments, we found several transcription units in the region between position -18 and -3 kilobases (kb), and one transcription unit between position 0 and +12.5 kb which gives rise to two messenger RNAs of 3.4 and 3.1 kb (Fig. 1d). The latter transcription unit has been identified as the *bib* gene because: (1) it overlaps with genomic alterations in different *bib* alleles (a deletion in *bib*¹¹⁹⁻⁵, an inversion breakpoint in *bib*^{C7a}, and a small deletion in *l(2)neo121*) as revealed by Southern blot analyses (Fig. 1b); (2) the P-element in *l(2)neo66* is inserted into the transcribed region of this transcription unit (at base pair (bp) 147 of a cDNA, c13, see below), whereas the insertion site in *l(2)neo91* is only 19 bp upstream of that cDNA; (3) introduction of a genomic DNA fragment including this transcription unit (PWB in Fig. 1c) by P-element mediated transformation^{18,19} rescued the neurogenic phenotype and lethality of *bib* mutants.

We have examined the distribution of *bib* mRNA by *in situ* hybridization to whole-mount embryos (Fig. 2). Expression of *bib* begins soon after the cell membrane starts to form around the nuclei in the syncytial blastoderm and is detectable in all the somatic cells that are forming in an early stage-5 embryo (staging is according to ref. 2). The *bib* mRNA soon disappears in a ventral region; the width of this region is about 10 cells at the stage of blastoderm formation when roughly one fifth of the cell membrane is formed (Fig. 2a) and increases to about 17 cells when cellularization is complete (late stage 5). This region of about 17 cells includes all presumptive mesodermal cells and two rows of ectodermal cells. During gastrulation at stages 6 and 7, the mesoderm invaginates, leaving one row of ectodermal

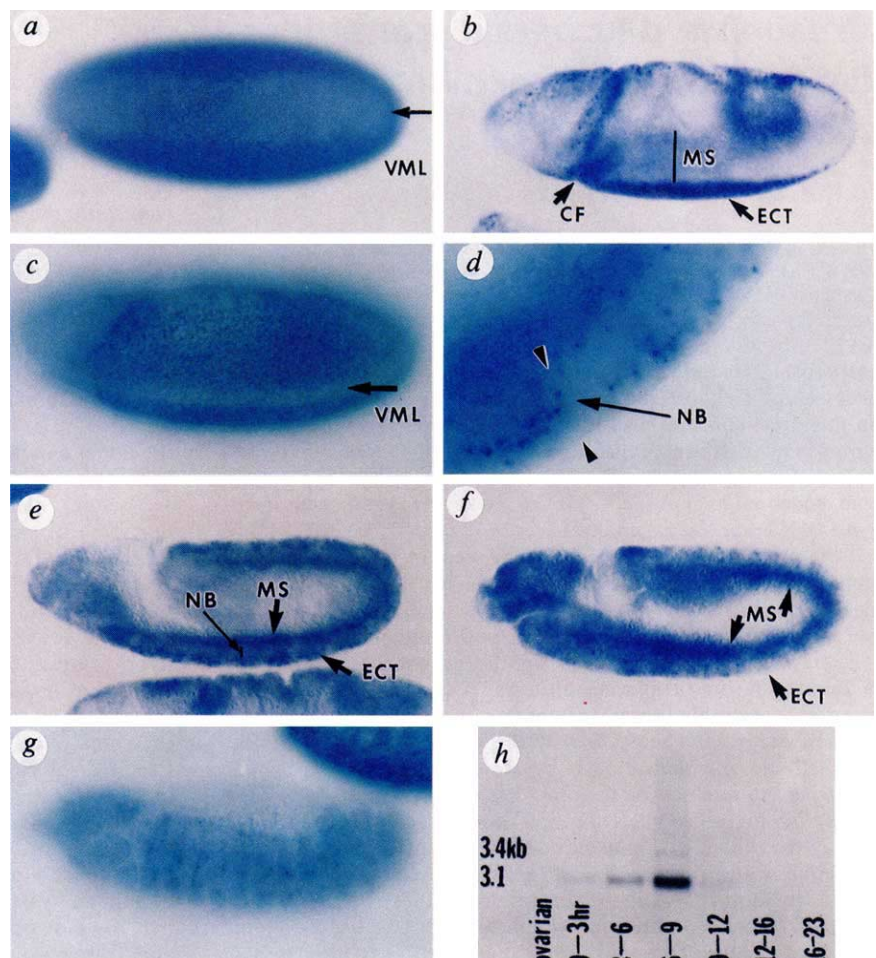
FIG. 1 Molecular map of *bib*. *a*, Restriction map of the *bib* genomic locus, as well as location of P-elements. Bg: *Bgl*I, Bm: *Bam*HI, E: *Eco*RI, H: *Hind*III, S: *Sal*I, X: *Xho*I. The *Eco*RI fragment at position 0–2.6 kb is polymorphic so that the second chromosome balancer, CyO (Curly derivative of Oster⁴⁰), has a 2.9 kb instead of a 2.6 kb fragment. The insertions of a P-element-derived vector pUChsneo in *l(2)neo66* and *l(2)neo91* are in opposite orientations. *b*, Location of X-ray induced mutations of *bib*: *bib*^{III9-5} has a deletion that includes the fragment indicated by the solid line and the ends could not be mapped in the cloned region; *bib*^{C7a} is an inversion allele with one of its breakpoints indicated by the solid line; *l(2)neo121* has a deletion in the region indicated by the solid line. *c*, PWB is a 15-kb DNA used in P-element mediated germ-line transformation. *d*, Poly(A)⁺ RNA panel shows the results of transcriptional analysis. The size of each transcript is indicated by the length of the line and the transcripts are positioned roughly under the genomic fragments that hybridize to them; e>I indicates that a transcript is more abundant in the early embryonic RNA (1–14 h) than in late embryonic RNA (8–21 h). Probes from position 1–2.6 kb and 2.6–3.2 kb hybridize to the same two messages of 3.4 and 3.1 kb. Position 8.7–12.3 kb contains repetitive sequences. *e*, cDNA panel shows c13, one of the longest of 17 cDNAs isolated with probes made from genomic fragments (1–3.2 kb); c13 hybridizes to both messages on northern blots and to genomic DNA fragments, at positions 1–2.6, 2.6–3.2 and 8.7–12.3 kb. The sequence of c13 contains *opa* repeats. METHODS. Poly(A)⁺ RNA used in northern blots was described previously³⁵. Embryonic cDNA libraries were from N. Brown³⁶. Insertion sites of pUChsneo in *l(2)neo66* and *l(2)neo91* have been determined by sequencing genomic fragments rescued from these lines. In addition to the deletion shown above,



l(2)neo121 has a P-element insertion at 49D on the right arm of the second chromosome. This insertion is not responsible for the *bib* phenotype because a recombination event which separated the two arms of the second chromosome in *l(2)neo121* revealed that the lethality segregated with the left arm rather than with the pUChsneo insertion on the right arm. Moreover, deficiency of 49D does not give rise to any neurogenic phenotype (unpublished observation).

FIG. 2 Embryonic expression of *bib*. Orientation of the embryos is anterior to the left (in *a*, *b*, *c*, *e*, *f*, *g*), and dorsal to the top (in *b*, *c*, *e*, *f*, *g*). Staging is according to ref. 2. No *bib* expression is seen before the cell membrane starts to form. *a*, Ventral view of a stage-5 embryo showing absence of expression in the presumptive mesoderm. VML indicates the ventral midline. *b*, Side view of a stage-7 embryo showing *bib* expression only in the ectoderm and not in the mesoderm during gastrulation. *c*, After ventral furrow formation, no *bib* mRNA is detectable in two rows of ectodermal cells, one row on each side of the ventral midline in an early stage-8 embryo; *d*, *bib* expression in a segregating neuroblast in a stage-9 embryo. Arrowheads point to the neuroblast which has its nucleus moved inward, but has its cell membrane still in contact with its ectodermal neighbours; *e*, *bib* expression in both the epidermal and the mesodermal layers but not in the neuroblast (NB) layer in a stage-10 embryo after germ-band elongation. *f*, In a stage-11 embryo, *bib* expression is still in the mesoderm, but not in the ectoderm. *g*, After germ-band shortening in a late stage-12 embryo, there are *bib* transcripts in a few cells per segment but the identity of these cells cannot be resolved in these embryos. *h*, Developmental northern analysis: no *bib* message is found in ovarian RNA. Numbers in the other lanes denote the age of the embryos from which the RNA was prepared.

METHODS. Whole-mount *in situ* hybridization³⁷ was carried out with a digoxigenin-labelled probe (template: genomic DNA at coordinate 0.9 to 8.7 kb). The staining is absent in *bib*^{III9-5} mutant embryos.



The result of developmental northern blot analysis correlates with the temporal expression pattern detected by *in situ* hybridization (Fig. 2h). Furthermore, no *bib* message is found in the ovary, consistent with the observation that *bib* is the only known neurogenic gene that has no detectable maternal contribution⁶.

We have analysed a possibly full-length cDNA, c13, which corresponds to the identified *bib* gene. The predicted 700 amino-acid *bib* product has some notable structural features (Fig. 3). Its N-terminal half is highly hydrophobic (Fig. 4a), whereas the C-terminal portion is generally hydrophilic and glutamine-rich. There are six hydrophobic stretches of more than 21 amino-acid residues (boxed in Fig. 3) that could potentially span the membrane²⁰. As there is no apparent signal peptide, both the N- and the C-termini may be intracellular.

The *bib* protein has significant sequence homology to the major intrinsic protein (MIP) of bovine lens fibre cell membrane¹⁰; 40% of the 224 amino acids (residues 56–279) in the N-terminal half are identical to those in MIP. This region also has sequence similarity to a soybean membrane protein, nodulin (nod) 26 (with 27% amino-acid identity)^{11,12}, and an *Escherichia coli* protein, GlpF (with 23% amino-acid identity)¹³ (Fig. 4b). The *bib* protein is about twice as large as the other proteins; the C-terminal half of *bib* shows no significant homology to any known proteins.

MIP is the most abundant membrane protein in mammalian lens fibre cells²¹. It is localized on the cell membrane^{22–25}. As the lens is avascular, cell-cell coupling is essential to ensure extensive metabolite exchange. There are abundant lens junctional structures between the fibre cells, some of which resemble gap junctions. It has been postulated that MIP is involved in

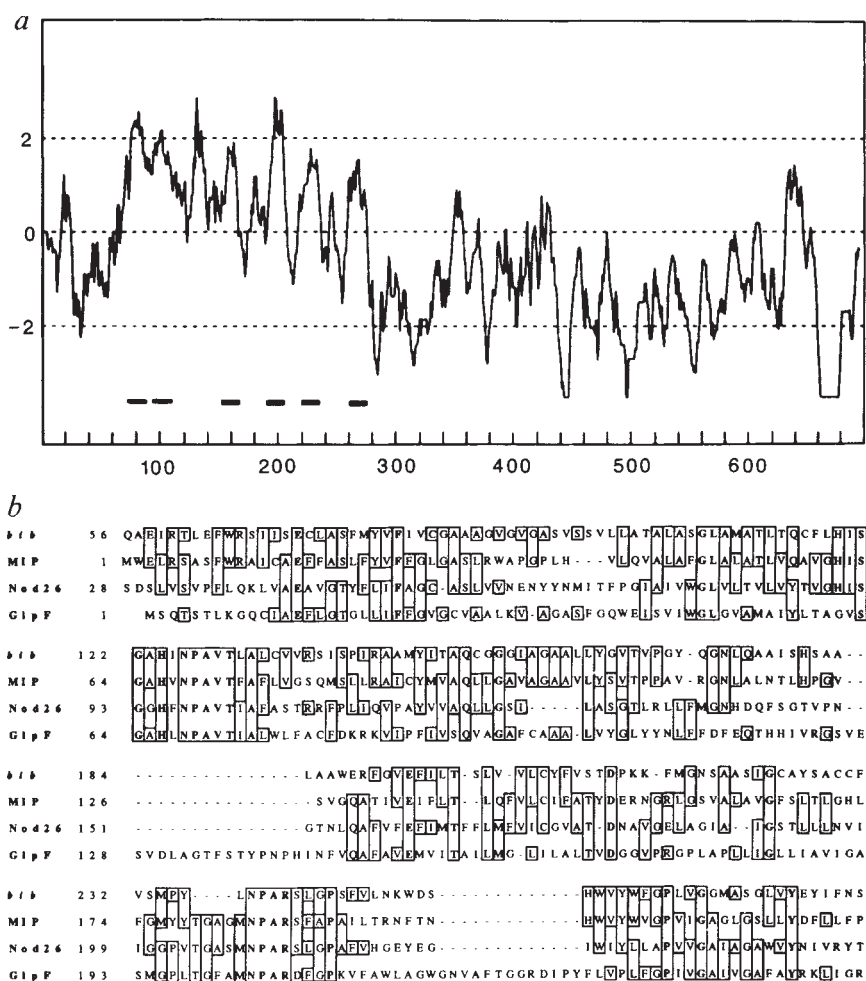
forming gap junctions between the lens fibre cells^{22,23,26}, but there is conflicting evidence. Unlike connexins, MIP has not been shown to form gap junctions, and ultrastructurally, MIP is localized not only to gap junctions but also to other types of junctions^{24,25}. Injection of MIP mRNA into *Xenopus* oocytes also failed to cause gap junction formation²⁷. However, MIP has been reconstituted into the lipid bilayer and shown to form non-selective channels with large conductance¹⁴.

Nod 26 is a transmembrane protein made by soybeans. In legume-*Rhizobium* symbiosis, after the bacteria infect the plant cells and are endocytosed into the cytoplasm, a series of events leads to the formation of nitrogen-fixation nodules. Rhizobia within the soybean cytoplasm are individually surrounded by the plant-derived peribacteroid membrane. There are about 20 proteins (the nodulins, including nod 26) made by soybean specifically for the peribacteroid membrane, which are believed to have important roles in morphogenesis and function of nitrogen-fixation nodules²⁸. Although the function of nod 26 is unknown, the peribacteroid membrane must be involved in regulating the exchange of nutrients and fixed nitrogen compounds between plant and bacteria.

GlpF is the glycerol facilitator of the *E. coli* inner membrane¹³. It is a passive channel permeable to neutral molecules smaller than 0.4 nm in diameter, including glycerol and glycine¹⁵. Although the *E. coli* outer membrane has several pore-type transporters, glpF is the only channel that allows bidirectional transport of molecules through the inner cytoplasmic membrane.

The sequence similarity between *bib*, glpF and MIP implies that the *bib* product may allow passage of small molecules across the membrane, which could mean that *bib* directly mediates

FIG. 4 Characterization of *bib* protein. *a*, Hydrophathy plot of the predicted *bib* protein. The Kyte and Doolittle program was run over 9-amino-acid stretches (the plot was similar when run with a window of 19 residues). The third and eighth hydrophobic peaks are not predicted by other programs as transmembrane domains, perhaps because there are several β -turns predicted in each region. The other six hydrophobic peaks indicated by the bars are boxed in Fig. 3 as the putative transmembrane domains. *b*, Sequence alignment of *bib*, MIP, nod 26 and glpF. The predicted 700-residue *bib* protein is longer than MIP (263 residues), nod 26 (271 residues) or glpF. The carboxy-half of *bib* contains many polyglutamine stretches and proline-rich regions and does not have significant sequence similarity with other proteins.



cell-cell interaction. Previous studies have suggested that, except for *bib*, the other known neurogenic genes belong to one pathway⁹. The products of two neurogenic genes, *Notch*^{29,30} and *Delta*³¹, are transmembrane proteins with epidermal growth factor-like repeats on their extracellular domains, that could directly mediate the intercellular interaction, whereas other genes in this pathway are either upstream (for example *neuralized*, or *mastermind*), or downstream (such as the *Enhancer of split* complex^{32,33}) of *Notch* and *Delta*. The *bib* product, however, may mediate intercellular communication in a manner independent of this pathway, as *bib* is the only neurogenic gene that shows no detectable genetic interaction with the other known neurogenic genes⁹.

Potentially, *bib* can mediate interaction either between neuroblasts and their surrounding ectodermal cells (the presumptive epidermoblasts), or among the epidermoblasts. In the former case, the *bib* mutant phenotype could result from the disruption of lateral inhibition exerted by the neuroblasts on their neighbouring cells. But if *bib* mediates interaction among epidermoblasts, such interaction could be essential for the presumptive epidermoblasts to stay in the epidermogenesis pathway rather than to take the default pathway of neurogenesis.

Results of a transplantation experiment³⁴ suggest that *bib* may act on the signalling side of cell-cell interaction. On the basis of the similarity between the *bib* protein and a number of channel proteins, we hypothesize that *bib* functions by allowing the release of certain molecule(s) and thereby sending a signal for an ectodermal cell to become an epidermoblast instead of a neuroblast. □

Abnormal sexual development in transgenic mice chronically expressing Müllerian inhibiting substance

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MÜLLERIAN inhibiting substance (MIS), also known as anti-Müllerian hormone, is a glycoprotein¹⁻⁴ normally secreted by the Sertoli cells of the fetal and adult testis^{5,6} and by granulosa cells of the postnatal ovary^{7,8}. The production of MIS in the male fetus brings about the regression of the Müllerian ducts, the anlagen of the uterus, oviducts, and upper vagina⁹⁻¹¹. In addition, purified MIS induces the formation of seminiferous cord-like structures in fetal rat ovaries cultured *in vitro*, suggesting that MIS may influence testicular differentiation¹². We have produced transgenic mice chronically expressing human MIS under the control of the mouse metallothionein-1 promoter to investigate its role during sexual development. In females, chronic expression led to the inhibition of Müllerian duct differentiation, resulting in a blind vagina and no uterus or oviducts. At birth the ovaries had fewer germ cells than normal; during the next two weeks germ cells were lost and the somatic cells became organized into structures resembling seminiferous tubules. Apparently, these structures degenerate as they are undetectable in adult females. The majority of transgenic males developed normally. But in two lines with the highest levels of MIS expression, some males showed feminization of the external genitalia, impairment of Wolffian duct development, and undescended testes. These results suggest that MIS has several distinct roles in mammalian sexual development.

We generated nine founder transgenic mice (two females and seven males) carrying a metallothionein-1 (MT)-MIS fusion gene (Fig. 1) by pronuclear injection of fertilized mouse eggs¹³. The MT promoter can direct expression of heterologous genes to a variety of fetal and adult tissues in transgenic mice¹⁴. Seven of the founders, including both females, had circulating levels

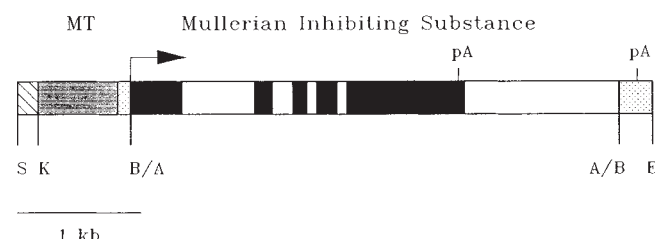


FIG. 1 Map of MT-MIS gene construct used to generate transgenic mice. The construct contains 650 base pairs (bp) of the mouse metallothionein-1 promoter (MT) (heavy shading) fused to the human MIS structural gene (exons, solid boxes; introns and 3' flanking region, open boxes). Mouse protamine-1 5' and 3' untranslated sequences are included (light shading) and 94 bp of simian virus 40 from *KpnI* to *SphI* (diagonal shading). S, *SphI*; K, *KpnI*; B, *BamHI*; A, *AflIII*; E, *EcoRI*; pA, polyadenylation signal. A 4,164-bp *AflIII* fragment containing the MIS structural gene was isolated from pBG311.hmis (ref. 3) and inserted into the *BamHI* site of the MTPpr.SVD expression vector. A 5-kbp *SphI-EcoRI* fragment was isolated from vector sequences and microinjected into fertilized mouse eggs as described¹³.

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TABLE 1 Summary of MT-MIS transgenic mice

Founder (sex)	Founder plasma hMIS (ng ml ⁻¹)	Progeny plasma hMIS (ng ml ⁻¹ (n; range))	Aberrant phenotypes observed (females, F; males, M)	Females analysed (n)	Males analysed (n)
1789-1 (M)	77	225 (9; 36-500)	F: no uterus, oviducts, ovaries*	9	9
1789-5 (F)	590	—	F: no uterus, oviducts, ovaries	1	—
1789-8 (M)	120	266 (12; 32-500)	F: no uterus, oviducts, ovaries	14	12
1789-9 (F)	147	—	F: no uterus, oviducts, ovaries	1	—
1790-7 (M)	0	—	—	—	—
1790-9 (M)	450	4,357 (7; 2,500-6,000)	F: no uterus, oviducts, ovaries*, M: undescended testes, feminization of external genitalia†	9	10
1791-3 (M)	0	—	—	—	—
1791-7 (M)	250	858 (17; 250-2200)	F: no uterus, oviducts, ovaries; M: undescended testes, feminization of external genitalia‡	11	11
1791-9 (M)	50	41 (17; 0-160)	F: no uterus, oviducts, ovaries§	19	9

Plasma human MIS levels were determined by an enzyme linked immunoabsorbent assay (ELISA)¹⁵. Phenotypes were observed in post-weaning offspring with the exception of the two sterile founder females.

* 1/9 females with bilateral ovaries.

† 3/10 males observed with undescended testes, underdeveloped epididymides, no seminal vesicles, vaginal opening, and mammary development.

‡ 2/11 males observed with undescended testes, underdeveloped epididymides, no seminal vesicles, vaginal opening, and mammary development; 2/11 males with small, yet properly descended testes.

§ 1/19 females was found to possess an ovary on one side but no uterus; 3/19 females with bilateral ovaries and no uterus; 3/19 normal females (uterus and ovaries present).

of human MIS ranging from 50–590 ng ml⁻¹ (Table 1). By comparison, MIS is undetectable in plasma from human adults¹⁵.

Both females were sterile; dissection revealed no uteri, oviducts, or ovaries. All five of the expressing founder males were fertile and transmitted the transgene to their progeny. Male and female progeny from each of the five established lines were characterized further for the presence of circulating human MIS and for any alterations in sexual development. Several founder males were germ-line mosaics and their progeny displayed a substantial increase in circulating levels of human MIS that ranged from 32 to 6,000 ng ml⁻¹ and varied between lines (Table 1). Variation was also observed in human MIS levels between individuals within a given line. The consequences of this variable expression were reflected in the different phenotypes detected between and within lines.

Nearly all female progeny that inherited the transgene, in all five lines, had a blind vagina and lacked uterus, oviducts, and ovaries (Table 1 and Fig. 2a). Variations were observed predominantly in the line expressing the lowest amount of MIS (1791-9), in which normal females or females lacking uteri but retaining ovaries were found (Table 1). Histological examination of the ovaries that persisted in transgenic females showed a range of ovarian morphology from normal to germ-cell deficient (data not shown). Thus, a higher level of MIS is apparently needed to produce the ovarian effect than is required for the Müllerian duct effect, since ovarian development can occur when MIS levels are sufficient to inhibit Müllerian duct differentiation.

To determine the stage at which ovarian development was being altered by chronic MIS expression, we bred transgenic males from the highest expressing line (1790-9) to non-transgenic females and identified female transgenic neonates by dot hybridization of tail DNA with transgene and male-specific probes¹⁶. Gonads were removed and processed for histological analysis (Fig. 3a–f). At 2 days after birth ovaries were present but no uterus had developed; the ovaries resembled controls, although germ cells were less prevalent. By 9 days after birth, the ovaries were devoid of germ cells and no follicles were present. The most dramatic effect of human MIS expression on ovarian tissue was observed 16 days after birth. The small gonads depleted of germ cells had developed cord-like structures bearing a striking resemblance to the seminiferous tubules of the

male gonad (Fig. 3f,g,h). No evidence of these structures was found in serial sections of ovarian fat pads of adult MT-MIS females, suggesting that these gonads degenerate with age.

The majority of transgenic males from all five lines were normal. But in the two highest expressing lines (1790-9 and 1791-7), 5 out of 21 of the transgenic males identified using male-specific probes had feminized external genitalia consisting of a vaginal opening and mammary gland development (Table 1 and Fig. 2b). These genetic males also possessed small, undescended testes devoid of germ cells, underdeveloped epididymides and no seminal vesicles (Fig. 3h). Feminization of

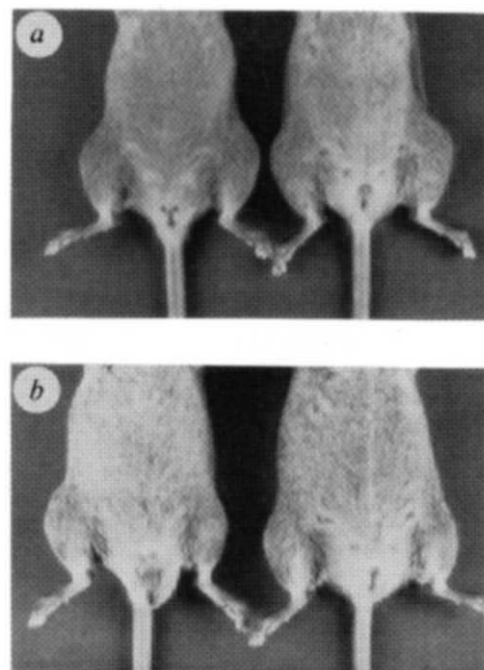


FIG. 2 External examination of a, female and b, male MT-MIS transgenic mice. In each panel the non-transgenic control is to the left and the transgenic is to the right.

the external genitalia and impairment of Wolffian duct development can be explained by androgen deficiency or insensitivity¹⁷⁻¹⁹. Apparently, high levels of MIS expression alter the development of the testes resulting in inhibition of androgen biosynthesis, perhaps by affecting Leydig cell differentiation and function. A perturbation in androgen synthesis cannot account for the inhibition of testicular descent, since the first phase of testicular descent is independent of androgen. Hutson and Donahoe have suggested that MIS may control this process, because a large percentage of patients with undescended testes have defective MIS or MIS receptor²⁰. Although the observations that both high and low levels of MIS can interfere with testicular descent seem to be contradictory, the high levels of MIS in the transgenic mice may lead to a down-regulation of the MIS receptor on the cells that control testicular descent.

In normal development, MIS is expressed in the gonads of both sexes, but with very distinct temporal patterns. The cell types that produce MIS, Sertoli cells in the testis and granulosa cells in the ovary, share many structural and functional characteristics and are believed to be derived from a common progenitor cell²¹. But expression of MIS in the Sertoli cell occurs early in fetal development of the male and after birth in the granulosa cells of the female. Expression of MIS in the Sertoli cell may be regulated by the testis-determining factor encoded by the Y chromosome because MIS is the first known product of Sertoli cells. Expression of MIS in the granulosa cell must involve a different mechanism.

In certain species, exposure of a female fetus to a male twin's blood by way of chorioallantoic anastomosis, results in regression of the Müllerian ducts, a condition known as freemartinism²². In addition, the ovaries cease to grow, become depleted of germ cells, and may develop seminiferous tubules containing Sertoli cells. As purified MIS can induce these changes in female fetal ovaries *in vitro*, it has been concluded that MIS is responsible for the ovarian freemartin effect^{12,23}. These observations have underscored the importance of silencing the MIS gene during female fetal development, and have suggested that MIS may be actively involved in testicular morphogenesis.

We have shown that chronic expression of MIS in female transgenic mice produces a phenotype similar to the freemartin. Müllerian duct differentiation is inhibited, resulting in a blind vagina and no uterus or oviducts, germ cells are lost, and eventually the somatic cells organize as seminiferous tubules. Unlike the freemartin condition, the ovary apparently degenerates by the adult stage. The nature of these effects suggests that MIS is interacting with both germ cells and somatic cells of the gonad. Although depletion of germ cells will prevent follicle formation and inhibit the postnatal development of the ovary, the loss of germ cells does not account for the formation of seminiferous tubules or the complete degeneration of the ovary, since these phenomena are not observed in W^v/W^v female mice depleted of germ cells²⁴. Rather, the formation of tubules and the eventual loss of the ovary is consistent with the direct effect of MIS on the granulosa cells of the female gonad. Together

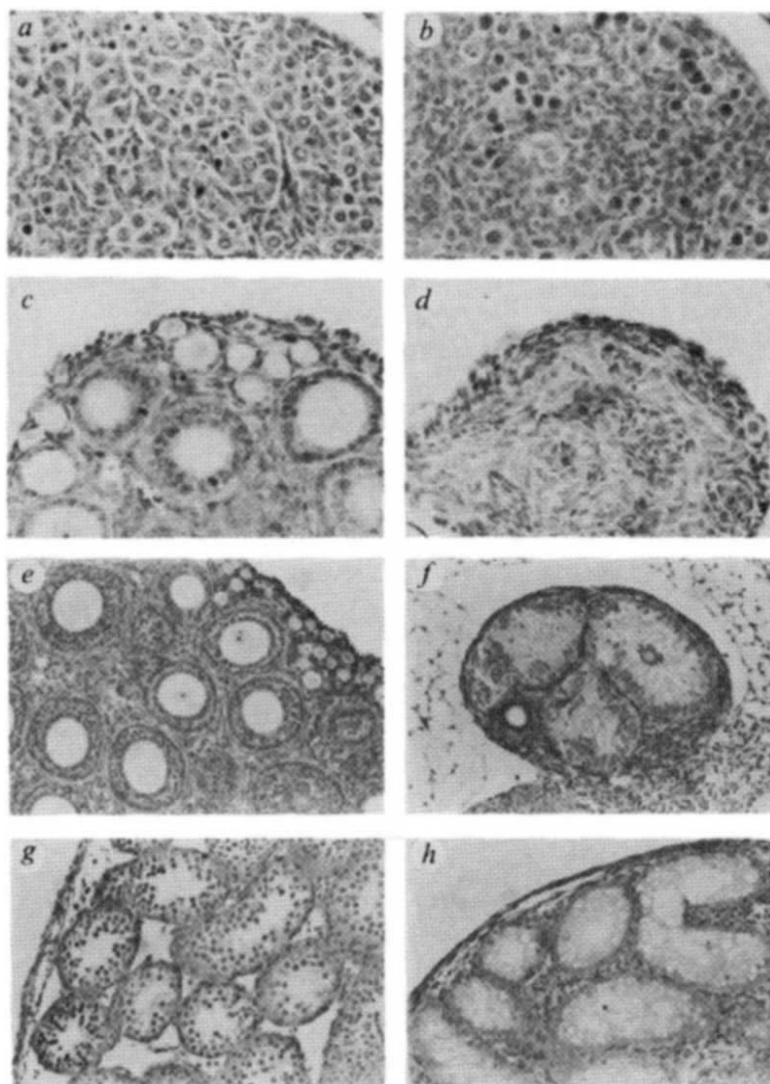


FIG. 3 Haematoxylin- and eosin-stained histological sections of control (a, c, e, g) and MT-MIS transgenic gonads (b, d, f, h). Female gonads postnatal day 2 (a, b), 9 (c, d), and 16 (e, f). Male gonads postnatal day 16 (g, h).

with the observation that high levels of MIS can perturb normal testicular development, these results provide further evidence that MIS is directly involved in testicular morphogenesis.

It should be noted that certain forms of male pseudohermaphroditism seem to be inconsistent with a role for MIS in testicular morphogenesis. Human males with persistent Müllerian duct syndrome retain a uterus, but possess testes which, although not properly descended, appear to be normal²⁵. It is possible that the altered MIS or MIS receptor responsible for this syndrome has a more pronounced effect on Müllerian duct development than on gonadal differentiation. The molecular analysis of MIS mutant phenotypes should clarify these issues.

The results of experiments with XX/XY chimaeras indicate that Sertoli-cell differentiation proceeds in a cell-autonomous manner through the action of the testis-determining gene (*Tdy*), but also requires an external inducing signal which is itself controlled by *Tdy*. Burgoyne *et al.*^{26,27} found that only XY cells become Sertoli cells in male XX/XY chimaeras, whereas both XX and XY cells contribute to the granulosa cell population in the relatively few XX/XY chimaeras that develop as female. In the latter case, ovarian development may proceed because the ratio of XY to XX cells is low, and the external inducing signal produced by XY cells is below the threshold level necessary for testis formation. As MIS is expressed early in the fetal testis and causes sex reversal of the neonatal ovary, it is a good candidate for this prospective external inducing substance.

The following model emerges to account for the role of MIS in testis formation. The testis-determining gene product(s) triggers Sertoli-cell differentiation and activates MIS gene expression. MIS then acts in an autocrine manner to induce further differentiation of the Sertoli cell. The Sertoli cell directs the subsequent steps of testis formation, including Leydig cell differentiation, germ cell proliferation, and seminiferous tubule formation. Because Sertoli and granulosa cells are thought to derive from a common progenitor and express MIS receptors, dysregulated expression of MIS in developing females leads to transdifferentiation of granulosa cells into Sertoli cells and the formation of seminiferous tubules. □

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Cryopreservation of *Drosophila melanogaster* embryos

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THERE is an urgent need to preserve the ever-increasing number (>30,000) of different genetic strains of *D. melanogaster* that are maintained in national and international stock centres and in the laboratories of individual investigators. In all cases, the stocks are maintained as adult populations and require transfer to fresh medium every two to four weeks. This is not only costly in terms of materials, labour and space, but unique strains are vulnerable to accidental loss, contamination, and changes in genotype that can occur during continuous culture through mutation, genetic drift or selection. Although cryopreservation of *Drosophila* germplasm would be an enormous advantage, many attempts using conventional procedures have been unsuccessful. *D. melanogaster* embryos are refractory to conventional cryopreservation procedures because of the contravening conditions required to minimize mortality resulting from both intracellular ice formation and chilling injury at subzero temperatures. To overcome these obstacles, we have developed a vitrification procedure that precludes intracellular ice formation so that the embryos can be cooled and warmed at ultra-rapid rates to minimize chilling injury, and have recovered viable embryos following storage in liquid nitrogen. In a series of 53 experiments, a total of 3,711 larvae emerged from 17,280 eggs that were cooled in liquid nitrogen ($18.4 \pm 8.8\%$). Further, using a subset from this population, approximately 3% of the surviving larvae (24/800) developed into adults. These adults were fertile and produced an F₁ generation.

There are two main impediments to the cryopreservation of *D. melanogaster* embryos: (1) the egg has a waxy layer that precludes water flux and the uptake of cryoprotectants into the embryo, and (2) the embryos are extremely sensitive to subzero temperatures. We have overcome the first obstacle by using sequential extraction with isopropanol and hexane to remove the waxy layer¹. Following this procedure, 80–90% of the eggs are permeable to both water and cryoprotectants (ethylene glycol, propylene glycol, glycerol, dimethyl sulfoxide). Most important, larvae emerge from 75–90% of the permeabilized eggs following culture in mineral oil.

Embryos of *D. melanogaster* are sensitive to chilling both at 0 °C and at subzero temperatures (<–10 °C), the degree of sensitivity varying with the stage of development^{2,3}. For example, with Oregon R Strain P2, the embryos are extremely sensitive to chilling at 0 °C during the first 6 h of the postoviposition period, and <20% of the eggs will hatch following a 3-h exposure at 0 °C³. At later developmental stages, the embryos are relatively insensitive to chilling at 0 °C; for example, 12- to 13-h old eggs can be maintained at 0 °C for up to 24 h with >90% of the eggs hatching. Nevertheless, even at this stage, the embryos are sensitive to temperatures below –10 °C; in supercooled eggs, survival declines to 11% after 30 min at –20 °C.

With conventional cryopreservation procedures, specimens are first equilibrated in molar concentrations of cryoprotectants and then the suspension is frozen to an intermediate subzero temperature (–30 to –40 °C) to effect freeze-induced cell dehy-

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dration. If the cytosol is sufficiently concentrated by cell dehydration, it will form a glass (vitrify) rather than crystallize during quenching in liquid nitrogen⁴. The cooling rate must be sufficiently slow (as determined by the rate of water efflux from the cells) to minimize supercooling of the cytosol and the incidence of ice formation. For *D. melanogaster* embryos, this rate is $<0.5^{\circ}\text{C min}^{-1}$ (refs 5, 6). However, if embryos are cooled at this rate, $<20\%$ survive cooling to -20°C and none survives to -35°C because of the prolonged periods at temperatures below -10°C , which result in a high incidence of chilling injury⁷.

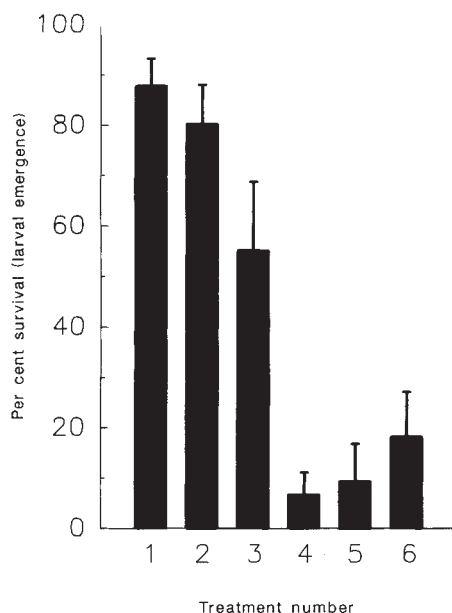


FIG. 1 Survival (emergence of larvae from the eggs) of *D. melanogaster* (Oregon R Strain P2) embryos following various treatments in the vitrification procedure. Values represent the means of a minimum of 30 observations, each with a minimum of 100 eggs. Error bars represent the standard deviation of the mean. Treatments: (1) Permeabilization. Eggs (13–14 h postoviposition, 60-min oviposition period) were permeabilized², placed in BD.20 (a modified basic culture medium for *Drosophila*¹⁰ containing 5 mM CaCl_2 , 9 mM MgCl_2 , 10 mM MgSO_4 , 3 mM NaH_2PO_4 , 68 mM glutamic acid, 67 mM glycine, 4 mM malic acid, 0.2 mM sodium acetate; pH 6.8, 260 mOsm) for 14 min, and cultured in mineral oil for 24 h at 25°C . (2) Loading with ethylene glycol. Eggs were permeabilized, equilibrated in BD.20 containing 2.125 M ethylene glycol plus 6% (w/v) bovine serum albumin for 20 min at 22°C and then diluted in BD.20 at 22°C and cultured as in treatment 1. (3) Dehydration in the vitrification solution. Eggs were permeabilized, loaded with 2.125 M ethylene glycol and then dehydrated in a vitrification solution (BD.20 containing 8.5 M ethylene glycol and 6% (w/v) bovine serum albumin) at 0°C for 8 min, then diluted in BD.20 and cultured. (4) Quenching eggs (in drops) in liquid propane. Eggs (permeabilized, loaded, and dehydrated) in 20- μl drops of the vitrification solution were expelled from a syringe into supercooled liquid propane and then the solidified droplets were transferred to liquid nitrogen for storage. Following removal from liquid nitrogen, the drops were rapidly transferred to BD.20 at 22°C for rapid warming and dilution of the vitrification solution, and the eggs were cultured as in treatment 1. (5) Quenching eggs (on copper grids) in liquid propane. Eggs (permeabilized, loaded, and dehydrated) were placed on copper grids (3 mm diameter, 0.8 mm thickness; Electron Microscopy Sciences), plunged in supercooled liquid propane, and transferred to liquid nitrogen. Following removal, the grids were transferred to BD.20 at 22°C as in treatment 4 and cultured as in treatment 1. (6) Quenching eggs (on copper grids) in liquid nitrogen slush. Eggs were treated as in treatment 5, except that they were plunged in liquid nitrogen slush rather than liquid propane. Slush was prepared by placing liquid nitrogen in a vacuum chamber to increase the rate of evaporation so that the temperature decreased to the freezing point (-209°C). The slush was used within 90 s following removal from the vacuum chamber, during which time the temperature of the fluid remained below -204°C .

If the embryos are cooled at faster rates (such as $3\text{--}6^{\circ}\text{C min}^{-1}$), about 40% survive cooling to -40°C . Cell dehydration is not, however, sufficient to prevent intracellular ice formation at lower temperatures and survival is precluded⁷. Until it is possible to ameliorate the subzero chilling sensitivity, conventional cryopreservation procedures are of little avail for *D. melanogaster* embryos.

In contrast, vitrification procedures⁸, which use highly concentrated solutions to dehydrate the specimens before quenching in cryogenic fluids and preclude ice formation in both the suspending medium and the specimen, allow for rapid cooling of specimens. For *D. melanogaster* embryos, such an approach would be expected to minimize chilling injury. To test this assumption, the vitrification procedure of Rall and Fahy⁹ was modified for *D. melanogaster* embryos. Permeabilized eggs were equilibrated in a cell culture medium (BD.20)¹⁰ containing 2.125 M ethylene glycol plus 6% (w/v) bovine serum albumin for 20 min at 22°C . Subsequently, the eggs were dehydrated and placed in 0.5 ml polypropylene straws and plunged into liquid nitrogen. During culture in mineral oil, larvae emerged from 87% of the permeabilized eggs (Fig. 1, treatment 1), and from 80% of the eggs equilibrated in ethylene glycol (Fig. 1, treatment 2). There were no viable larvae after recovery of the eggs from liquid nitrogen. The lack of survival was not attributable to the toxicity of the vitrification solution or removal of ethylene glycol from the embryos by direct dilution in BD.20 solution because larvae emerged from $\sim 55\%$ of eggs that were similarly treated but not plunged in liquid nitrogen (Fig. 1, treatment 3). Although the lack of survival was associated with the cooling/warming cycle, differential scanning calorimetry studies and visual inspection of the straws indicated that the solution vitrified during cooling and did not devitrify (crystallize) during warming.

Although routine, the use of polypropylene straws as a specimen container and liquid nitrogen as the cryogenic fluid results in a relatively slow cooling rate, because vaporization of the liquid nitrogen around the straw impedes heat transfer¹¹. With this procedure, the cooling rate (as measured with a 0.12 mm copper-constantan thermocouple and an oscilloscope) was initially slow ($\sim 15^{\circ}\text{C s}^{-1}$) over the range 0 to -60°C ; increased to $\sim 50^{\circ}\text{C s}^{-1}$ between -60 and -120°C , and then decreased at lower temperatures. The measured warming rate was $\sim 60^{\circ}\text{C s}^{-1}$ over the range of -196 to -100°C , and $\sim 16^{\circ}\text{C s}^{-1}$ over the range of -100 to -44°C . To improve heat transfer, the straws were plunged into liquid propane supercooled with liquid nitrogen. Although the cooling rate was greater ($\sim 55^{\circ}\text{C s}^{-1}$ from 0 to -60°C), there was no measurable survival of the eggs.

As an alternative procedure to increase the cooling/warming rate, the eggs (contained in $\sim 20\text{-}\mu\text{l}$ drops of the vitrification solution) were expelled directly from a syringe into liquid propane. In this way, the first successful recovery of viable eggs from liquid nitrogen was achieved (larvae emerged from $6.8 \pm 4.4\%$ of the eggs) (Fig. 1, treatment 4). Because it was difficult to control the size of the droplet, the procedure was modified so that the eggs were placed on a copper grid (~ 25 eggs per grid), which was forcibly propelled into liquid propane. The measured cooling rate was $\sim 700^{\circ}\text{C s}^{-1}$ from 0 to -60°C . Following removal from liquid nitrogen, the grids containing the eggs were transferred to BD.20 at 22°C to warm the eggs ($>300^{\circ}\text{C s}^{-1}$) and dilute the vitrification solution. During subsequent culture in mineral oil, larvae emerged from $9.4 \pm 7.5\%$ of the eggs (Fig. 1, treatment 5). In several instances, survival was $>20\%$; and, in one instance, a high of 27% was attained. But the results were extremely variable, possibly as a result of permeation of propane into the embryos. To eliminate this possibility, liquid nitrogen slush was used as a cryogenic fluid. Although the cooling rate during quenching ($400^{\circ}\text{C s}^{-1}$ from 0 to -60°C) was slower than that attained with liquid propane, the rate was greater than that achieved using liquid nitrogen ($15^{\circ}\text{C s}^{-1}$ for eggs in straws; $50^{\circ}\text{C s}^{-1}$ for eggs on grids) because

vaporization of the liquid nitrogen around the sample was minimized. A higher and more consistent percentage of larvae emerged from the eggs (3,711 larvae out of 17,280 cryopreserved eggs; $18.4 \pm 8.8\%$) (Fig. 1, treatment 6). This value includes the results from a study in which the embryos were dehydrated in the vitrification solution for different periods of time ranging from 5–8 min before quenching in liquid nitrogen slush. For the individual treatments, larval emergence was $18.7 \pm 9.2\%$, $20.0 \pm 10.3\%$, $17.3 \pm 8.1\%$, and $16.4 \pm 7.5\%$ for dehydration times of 5, 6, 7 and 8 min, respectively.

There are three possible reasons why viable embryos were recovered only with the ultra-rapid cooling and warming rates: (1) at slower cooling rates, a partially crystallized glass¹² formed within the embryo and/or the stability of the amorphous state was less¹³, such that devitrification occurred during warming at slower rates; (2) the incidence of chilling injury was minimized because of differences in the time at subzero temperatures; (3) if the embryos are sensitive to cold shock, defined as the mortality that occurs as a result of rapid cooling¹⁴, this may be responsible for the difference in survival between the procedures—assuming that cold shock is decreased at ultra-rapid cooling rates. Studies to resolve these possibilities are in progress.

We have used the emergence of larvae as the first criterion of survival in optimizing the various steps in the vitrification procedure. Given the numerous interacting variables in the method and the lack of information on the cryobiology of *D. melanogaster* embryos, our first priority was to obtain a consistent percentage of viable embryos following storage in liquid nitrogen. We then initiated studies to recover adults from the cryopreserved embryos, which has required the development of special culture procedures for both the embryos and the larvae. To attain the maximum number of emerging larvae, the permeabilized eggs must be maintained in mineral oil because of the extreme sensitivity of the embryos to desiccation. For continued development, the larvae must be transferred to a food source. But, if newly emerged larvae from permeabilized/cryopreserved eggs are directly transferred to an agar-based medium, few will survive. Pupariation of the larvae has been attained by transferring the newly emerged larvae to an aqueous glucose-yeast medium (3.75g yeast per 100 ml of a solution containing 114 mM glucose, 7.4 mM H_3PO_4 and 1.0 mM propionic acid; pH 5.0), the volume of which is controlled so that their trachea can protrude from the liquid. The larvae are maintained in this medium (with daily transfers to fresh medium) until the third instar, at which time they are transferred to a solid medium for pupariation. In preliminary studies, ~3% (24 out of 800) of the larvae that emerged from cryopreserved eggs developed into adults. The large decrease in survival occurs during larval development before pupariation, as more than 90% of the pupae develop into adults. The adults were fertile and produced an F_1 generation. Although it is necessary to increase the percentage of adults recovered from cryopreserved embryos, establish viability and genetic stability after long-term storage, and test the procedure with other strains before the procedure will be of practical use, these studies demonstrate the feasibility of cryopreservation of *D. melanogaster* embryos. □

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Invariant chain influences the immunological recognition of MHC class II molecules

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RECENT experiments have implicated intracellular events in the formation of the MHC class II-peptide complexes recognized by CD4-positive T cells^{1–6}. These data raise the possibility that the intracellular association of class II with the non-polymorphic glycoprotein, invariant chain (Ii)^{7,8}, may regulate the interaction between processed antigen and MHC class II molecules. To address this possibility, we have generated a series of transfected fibroblast cell lines that express class II with and without Ii. Although the presence of Ii does not seem to affect the ability of the cells to process and present intact antigen, Ii-negative cells express an altered form of class II at the cell surface. This modified conformation of class II in Ii-negative cells is detectable by an increase in the ability to present antigenic peptides to T cells and a decrease in the binding of several class II-specific monoclonal antibodies.

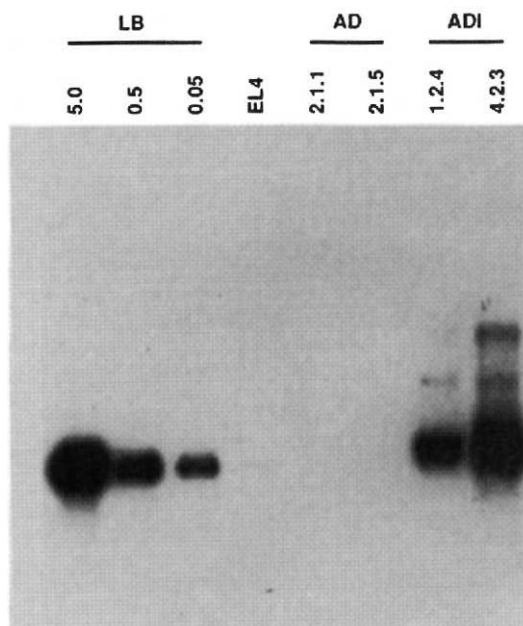
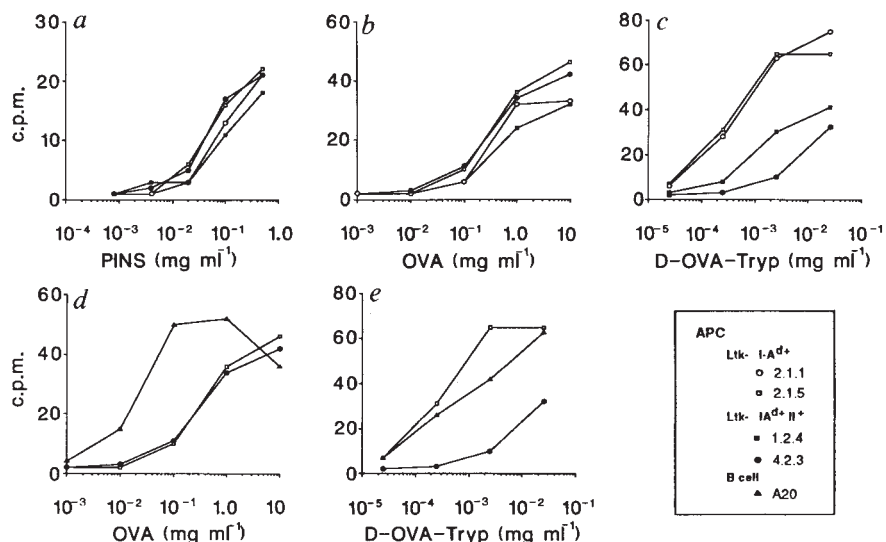


FIG. 1 Invariant chain is expressed in Ltk⁻ cells only after gene transfer. Cytoplasmic RNA (5.0 μg) from individual clones of Ltk⁻ transfectants expressing I-A^d alone (AD) or I-A^d plus Ii (ADI) were electrophoresed on a 1% paraformaldehyde gel, transferred to nitrocellulose, and hybridized to a radioactively labelled Ii probe as described in ref. 9. All lanes contained equal amounts of RNA as indicated by ethidium bromide staining and visualization of the ribosomal RNA bands in each sample. For controls, varying amounts (5.0, 0.5, and 0.05 μg) of RNA from the Ii-positive B-cell line, LB, and 5.0 μg of RNA from the Ii-negative T-cell line, EL-4, were run in parallel.

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FIG. 2 Peptide presentation is enhanced in the absence of Ii. Ltk⁻ transfectants expressing I-A^d, 2.1.1 (○) and 2.1.5 (□), or I-A^d and Ii, 1.2.4 (■) and 4.2.3 (●), were assayed for their ability to present various doses of antigen to I-A^d-restricted T-cell hybridomas. *a*, B8/C3.X T cells¹⁸ are specific for pork insulin (PINS). *b-e*, 3D0-54.8 T cells¹² are specific for chicken ovalbumin (OVA) and recognize a tryptic peptide derived from OVA (D-OVA-Tryp) without the need for additional processing. *d-e*, Comparison of the presentation efficiency of antigen (*d*) and peptide (*e*) between the Ltk⁻ transfectants and the class II-positive, Ii-positive B-cell line, A20 (▲). Identical results to the D-OVA-Tryp assays were seen with purified OVA₃₂₃₋₃₃₉ peptide. Data represent the incorporation of ³H-thymidine (c.p.m. × 10³) by the IL-2-dependent T-cell line, CTLL, when cultured in the presence of 25% culture supernatant from the antigen presentation assays.



Although the expression of Ii does not affect the efficiency of class II cell-surface expression (Table 1 and refs 9-11), it may affect the intracellular transport events associated with class II-peptide association. To address this possibility, class II-positive transfectants that either do or do not co-express Ii (Table 1 and Fig. 1) were compared for their ability to present antigen to antigen-specific, MHC-restricted T-cell hybridomas. Expression of Ii did not influence the ability of the transfectants to process and present intact antigen, although both Ii-positive and Ii-negative transfectants presented antigen less efficiently than the B-cell line, A20 (Fig. 2). Surprisingly, when the transfectants were tested for their ability to present antigenic peptides, the Ii-negative cell lines presented 10-50 times better than the Ii-positive cells (Fig. 2c). Thus, it seems that the absence of Ii enhances the ability of cells to present antigenic peptides, without influencing the ability of the same cells to present intact antigen to the same T-cell hybridoma.

The predominantly intracellular association of class II and Ii

suggests that Ii may exert an effect on antigen presentation only after endocytosis of the antigen. To determine whether the reduced efficiency of peptide presentation by Ii-positive cells was due to an intracellular event, the transfectants were fixed with paraformaldehyde before assay. Fixation does not affect the enhanced presentation of peptides in the absence of Ii (Fig. 3). Fixation was complete as evidenced by the inability of the fixed cells to present intact ovalbumin, an antigen which requires internalization and processing¹². Thus, it appears that the enhanced presentation of peptide in the absence of Ii is a stable cell-surface phenotype.

An influence of Ii on cell-surface peptide presentation could be due to direct participation in cellular interactions or to an indirect effect on the conformation of class II. To test for an alteration in class II structure, Ii-positive and Ii-negative transfectants were stained with a panel of class II-specific monoclonal

TABLE 1 Transfected cell lines

Cell line	Transfected DNA	Expression levels	Ii
2.1.1	I-A ^d	62	0
2.1.5	I-A ^d	54	0
1.2.4	I-A ^d +Ii	93	0.1
4.2.3	I-A ^d +Ii	23	1.0

Ltk⁻ fibroblast cells (ATCC) were co-transfected with complementary DNA clones encoding A_α^d and A_β^d (ref. 9) to generate clones 2.1.1 and 2.1.5 or with cDNA clones encoding A_α^d, A_β^d (ref. 9) and Ii (ref. 17) to generate clones 1.2.4 and 4.2.3. Cells resistant to neomycin (G418, Gibco) were positively sorted for I-A^d expression with magnetic beads (Dynal) and cloned by limiting dilution. Individual clones were assayed for class II expression by flow cytometry after staining with 34.5.3 and FITC-GAM (see Fig. 4). Values for I-A^d expression represent mean fluorescence intensity. The B-cell line, A20, stained in parallel, had a mean fluorescence intensity of 107. The transfectants were assayed for Ii expression by northern blot analysis. Values represent relative Ii messenger RNA levels compared to the B-cell line, LB (see Fig. 1). We occasionally observed the induction of low levels of endogenous Ii expression in untransfected Ltk⁻ cells grown to high densities. All experiments in this report were therefore performed on cells grown at low density and Ii RNA was not detectable in untransfected cells at the time of the APC assay (see Fig. 1). In addition to the cells, listed above, we have examined four independent clones expressing either I-A^d or I-A^dIi. All cells examined express the same Ii-dependent phenotypic differences described below and, therefore, these effects cannot be attributed to clonal variation independent of Ii expression.

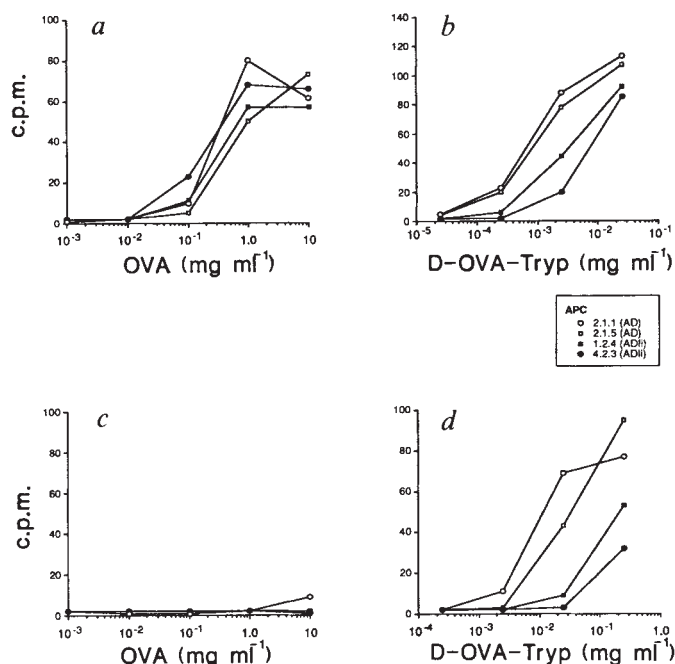


FIG. 3 Enhanced presentation of peptide in the absence of Ii represents a cell-surface phenomenon. Presentation of intact antigen (OVA) or peptide (D-OVA-Tryp) to 3D0-54.8 T cells before (*a* and *b*) and after (*c* and *d*) fixation with 1% paraformaldehyde. Antigen presentation assays were performed as in Fig. 2. Symbols as in Fig. 2.

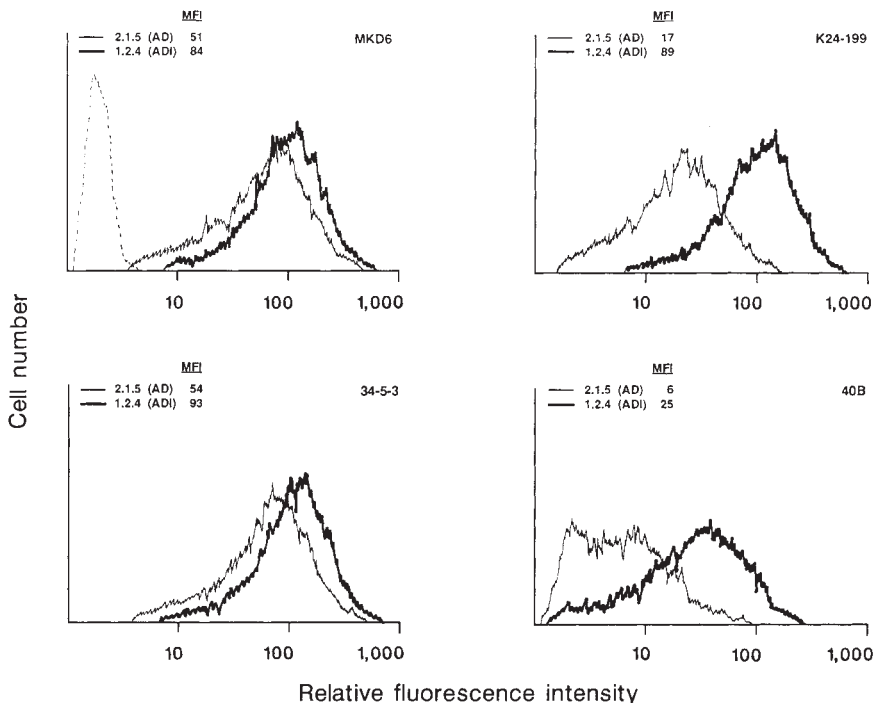


FIG. 4 Conformation of class II at the cell surface is altered in the absence of Ii. Ltk⁻ cells transfected with I-A^d (2.1.5) (—) or with I-A^d and Ii (1.2.4) (---) were stained with a panel of monoclonal antibodies to I-A^d as described in Table 1. MKD6 (ref. 19), 34-5-3 (ref. 20), M5/114 (ref. 21), and 25-7-17 (ref. 22) do not distinguish between Ii-positive and Ii-negative cells; whereas K24-199 (ref. 23), 40B (ref. 24), and 39H (ref. 24) bind less well to I-A^d expressed on Ii-negative cells. The dashed line in a represents cells stained with the secondary FITC-GAM reagent only. MFI, mean fluorescence intensity.

antibodies. Strikingly, several antibodies, K24-199, 40B (Fig. 4), and 39H (not shown), bound at significantly reduced levels on Ii-negative cell lines, whereas other antibodies, MKD6, 34-5-3 (Fig. 4), 25-9-17, and M5/114 (not shown), were not affected. Thus, expression of Ii is required for the generation of some class II-specific antibody epitopes at the cell surface. Together with the effects of Ii on peptide presentation, these results suggest that expression of Ii controls structural features of class II relevant for immunological recognition.

Because the majority of class II expressed at the cell surface is no longer associated with Ii, the effect of Ii on the conformation of class II is probably imparted indirectly during biosynthesis. In light of the dramatic effects of peptide association on class I expression¹³, we have attempted to rescue normal class II epitope expression by incubation of the Ii-negative transfectants with exogenous, I-A^d-binding peptides. This procedure did not alter the level of K24.199 or 40B staining (M.P. and J.M., unpublished results). Our preliminary data also suggest that in Ii-negative cells, the reduced staining of 40B and K24.199 represents a lower affinity of these antibodies for class II rather than a decrease in the number of class II molecules that express the epitopes. Taken together, these results suggest that the diminished binding of these antibodies in Ii-negative cells is not simply due to the failure of a subset of class II molecules to associate with peptide. A loss of reactivity with a subset of class II-specific monoclonal antibodies has recently been detected in mutant cell lines that seem to be defective in antigen presentation¹⁴. Interestingly, these cells express normal amounts of Ii (ref. 14), suggesting that similar alterations in class II structure can result from different biochemical defects.

The data presented here differ from previous analyses of L cell transfectants. DAP-3 L cell transfectants express class II molecules that are structurally and immunologically indistinguishable from those expressed by B-cell lines¹⁵. These studies do not address the role of Ii because the recipient L-cell line, DAP-3, is Ii positive. Stockinger *et al.*¹⁶ have recently reported that transfection of Ii into class II-positive, Ii-negative Ltk⁻ transfectants significantly enhanced the capacity of these cells to process and present intact antigen. Although additional studies will be required to resolve the conflict between our results and those of Stockinger *et al.*, one possible interpretation is that

T-cell recognition of the antigens used in their study may require processing events which are dependent on the presence of Ii, whereas the immunodominant ovalbumin and insulin epitopes examined in this report may not.

Several functions have been suggested for Ii in class II-restricted antigen presentation. Ii could occupy the antigen-binding site early in the biosynthetic pathway, protecting class II from associating with endogenous peptides in the endoplasmic reticulum. Alternatively, Ii could modify class II structure by altering its folding, post-translational processing, or intracellular transport. Finally, Ii may influence the efficiency of class II-peptide association in an endosomal compartment. Although our results do not discriminate between these possibilities, they do show that Ii significantly affects the conformation of class II at the cell surface. We are currently designing experiments to determine the biochemical basis and subcellular site(s) in which Ii influences class II structure and function. □

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***Bacillus subtilis* expressing a haemolysin gene from *Listeria monocytogenes* can grow in mammalian cells**

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INTRACELLULAR parasites can be classified into those that reside within a host vacuole and those which grow directly in the host cytoplasm¹. Members of the latter group, which includes *Rickettsia*², *Shigellae*³, *Trypanosoma cruzi*⁴, and *Listeria monocytogenes*^{5,6}, possess haemolytic activity associated with the ability to enter the host cytoplasm⁵⁻⁷. Therefore mutants of *L. monocytogenes* lacking a pore-forming haemolysin, listeriolysin O, do not escape from the endosomal compartment^{5,6} and consequently fail to become established in the cytoplasm^{5,8,9}. To examine the role of listeriolysin O, we cloned the structural gene for the *L. monocytogenes* haemolysin, *hlyA*, into an asporogenic mutant of *Bacillus subtilis* under the control of an IPTG-inducible promoter¹⁰. After being internalized by the macrophage-like cell line J774, haemolytic *B. subtilis* disrupted the phagosomal membrane and grew rapidly within the macrophage cytoplasm. These results show that a single gene product is sufficient to convert a common soil bacterium into a parasite that can grow in the cytoplasm of a mammalian cell.

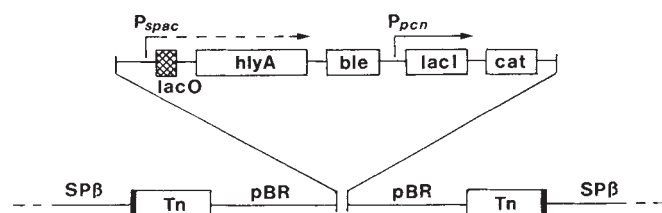


FIG. 1 Physical map of sequences integrated into the *B. subtilis* chromosome that place expression of the *L. monocytogenes* *hlyA* gene under control of the IPTG-inducible P_{spac} promoter. Cross-hatched box (*lacO*), repressor-binding sequences from the *Escherichia coli* *lac* operator region; P_{spac} , strong promoter derived from *B. subtilis* phage SP01 whose expression is constitutive except when *lac* repressor is bound to *lacO*; *hlyA*, DNA fragment containing the entire coding sequence of listeriolysin O but lacking a promoter that can function in *B. subtilis*; *ble*, gene conferring resistance to bleomycin and bleomycin in *B. subtilis*; P_{pcn} , constitutive promoter derived from a *B. licheniformis* penicillinase gene; *lacI*, promoterless copy of the *E. coli* *lac* repressor gene; *cat*, gene conferring chloramphenicol resistance in *B. subtilis*; pBR, sequences derived from plasmid pBR322; Tn, sequences derived from transposon Tn917; SPB, prophage of *B. subtilis* temperate phage SP β c2del2::Tn917; solid arrow, constitutive transcription directed by P_{pcn} ; arrow with broken line, IPTG-inducible transcription directed by P_{spac} .

METHODS. A 2.5-kb *Sau*96 1 fragment of *L. monocytogenes* chromosomal DNA containing the *hlyA* coding sequence¹¹ was inserted by ligation into the *Hind*III site of pAG58-*ble*-1 (ref. 10), in an orientation appropriate for transcription of the *hlyA* gene by P_{spac} . DNA was treated with Klenow fragment to produce flush ends before ligation. Constructions containing the insert were integrated into the chromosome of *B. subtilis* by transformation of an asporogenic derivative of strain ZB307 (ref. 16), selecting for phleomycin resistance. The pBR322-derived sequences in both pAG58-*ble*-1 and the SP β prophage in ZB307 provided homology for integrative recombination.

The structural gene for the *L. monocytogenes* haemolysin, *hlyA*, was cloned on a 2.5-kilobase (kb) *Sau*96 1 fragment¹¹ into the *Hind*III site of plasmid pAG58-*ble*-1 downstream from the IPTG-inducible SPAC cassette¹⁰. This expression cassette was then integrated into the chromosome of a nonreverting sporulation-deficient mutant of *B. subtilis* by homologous recombination (Fig. 1). The resulting strain, *B. subtilis* (*hlyA*), secreted haemolytic activity only during growth in the presence of IPTG (data not shown). After induction, this strain secreted about the same haemolytic activity (80 units ml⁻¹) as wild-type *L. monocytogenes* grown in the same conditions. A new polypeptide was also secreted only after IPTG-induction and co-migrated with authentic listeriolysin O (of relative molecular mass (M_r) 58,000)¹² as seen by SDS-PAGE (data not shown). These data indicate that a single gene product is sufficient for production and secretion of haemolytic activity by *B. subtilis*, and that this activity depends on induction of the SPAC promoter.

To investigate the role of haemolysin in establishment of intracellular growth we challenged the phagocytic macrophage-like cell line J774 with *B. subtilis* (*hlyA*). Extracellular multiplication was prevented by the addition of gentamicin after 1 h. To our surprise, *B. subtilis* (*hlyA*), when grown and used to infect the cells in the presence of IPTG, multiplied inside the macrophages with an intracellular doubling time of about 1 h (Fig. 2). This is the same intracellular doubling time as *L. monocytogenes* (ref. 8 and Fig. 2). In the absence of IPTG, *B. subtilis* was internalized and killed to some extent, and no growth was detectable (Fig. 2). In addition, IPTG could be added after internalization ($t = 2$) and the surviving intracellular bacteria commenced growth (data not shown). This suggests that bacteria residing in a vacuole were capable of *de novo* protein synthesis

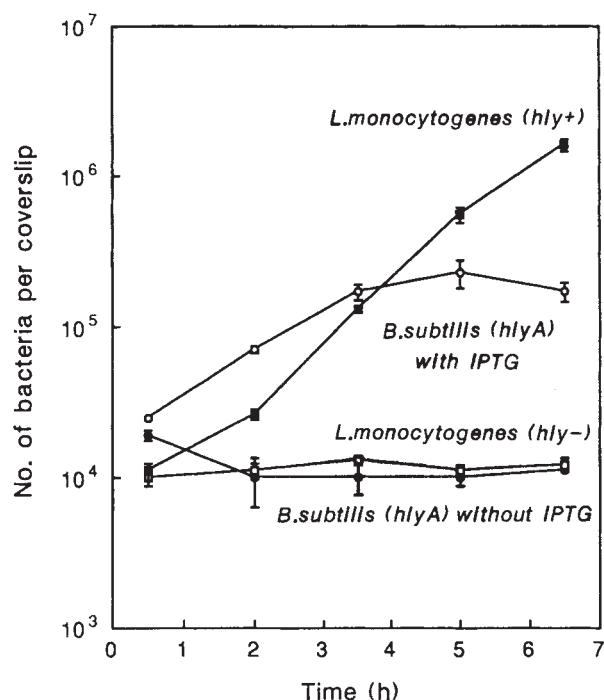


FIG. 2 Growth of *hly*⁺ and *hly*⁻ *L. monocytogenes* and *B. subtilis* in the J774 macrophage-like cell line. *Hly*⁺ *L. monocytogenes* (■); *hly*⁻ *L. monocytogenes* (□); *B. subtilis* (*hlyA*) with IPTG (○); and *B. subtilis* (*hlyA*) without IPTG (●). METHODS. Growth of *L. monocytogenes* in J774 cells has been described previously⁸. *B. subtilis* was grown at 37 °C to an optical density of 0.5 at 600 nm with or without 10 mM IPTG. J774 cells grown on coverslips in a 60-mm petri dish in a volume of 5 ml tissue culture medium were infected with 5 μ l *B. subtilis* culture with or without 10 mM IPTG. After 30 min, the monolayers were washed three times with 37 °C PBS (pH 7.4) and fresh medium was added without IPTG. All other procedures have been described elsewhere⁸. Error bars represent standard deviation of bacteria per coverslip in triplicate.

FIG. 3 Light micrographs of *hly*⁺ *B. subtilis* growing in J774 cells. *a*, Two hours after infection (three bacteria); *b*, five hours after infection (>50 bacteria). Infection of J774 cells was as described in Fig. 2. Coverslips were stained with Diff-Quik (Baxter Healthcare).

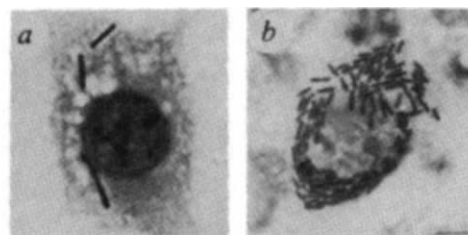
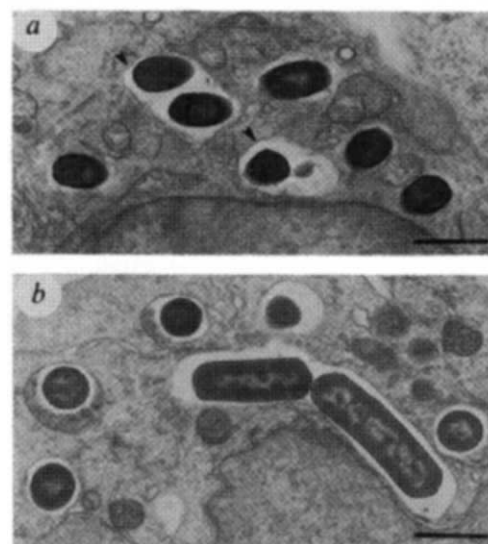


FIG. 4 Electron micrographs of *B. subtilis* in J774 cells. *a*, *B. subtilis* (*hlyA*) with IPTG. (Magnification, $\times 13,000$). Three of the bacteria are in phagosomes (arrows) while the other four are free in the cytoplasm. *b*, *B. subtilis* (*hlyA*) without IPTG. All the bacteria are in phagosomes. ($\times 10,000$). Bar, 1 μ m. Infection of J774 cells was as described in Fig. 2 but 10 times the number of bacteria were added per ml. After 2.5 h infection, samples were fixed *in situ*, embedded, thin sectioned, and observed by electron microscopy as described previously⁶.



even though they were unable to multiply. Cells were also challenged under the same conditions with *B. subtilis* harbouring the vector alone, and no bacterial growth was observed (data not shown).

Light microscopy of stained preparations revealed extensive intracellular multiplication by the haemolytic *B. subtilis* strain (Fig. 3). But unlike *L. monocytogenes*, which spreads to the periphery of infected cells and then from cell to cell^{6,13}, haemolytic *B. subtilis* showed no evidence of such cell to cell spread and grew until the infected host cell eventually lysed. This was reflected in the decrease in colony-forming units seen at later times (Fig. 2). Electron microscopy revealed that some of the haemolytic *B. subtilis* were clearly growing freely in the host cytoplasm, whereas others appeared intact in vacuoles (Fig. 4*a*). By contrast, all of the *hly*⁻ *B. subtilis* were in vacuoles (Fig. 4*b*). These data indicate that haemolysin alone mediates lysis of the host vacuole, although it cannot be ruled out that another *B. subtilis* gene product acts with the *L. monocytogenes* haemolysin.

Haemolytic *L. monocytogenes* normally grows in the macrophage cytoplasm surrounded either by a cloud or a long tail of actin filaments, and this is thought to be a necessary prerequisite for cell-to-cell spread⁶. But it is still not known whether bacteria or host mediates actin filament polymerization. It was therefore of interest to determine whether *B. subtilis* growing in the host cytoplasm becomes associated with host actin filaments. Electron micrographs show that intracytoplasmic *B. subtilis* grow 'naked', without actin filaments (Fig. 4*a*). These results indicate that the expression of haemolysin is sufficient to mediate access to the host cytoplasm, but other, so far undefined, *L. monocytogenes* gene products are required to nucleate actin polymerization.

Although it was not unexpected that haemolytic *B. subtilis* could lyse the phagosomal membrane, it was surprising that the bacteria grew within the cytoplasm. Thus, a single gene product is sufficient to convert a common soil organism into an intracellular parasite *in vitro*. This implies that the mammalian cytoplasm is a very permissive environment for bacterial growth. In contrast to the situation *in vitro*, haemolytic *B. subtilis* was

absolutely avirulent after intravenous injection in BALB/c mice (D. Hinrichs, personal communication). This is consistent with the multifactorial nature of bacterial pathogenicity¹⁴. Clearly, one gene does not convert an organism into a pathogen. Nevertheless, the results of this study indicate that the evolutionary leap from an extracellular existence to an intracellular life-style may only require the acquisition of a limited number of genes.

Haemolytic activity has been observed in a wide variety of viral, bacterial and protozoan pathogens. The haemolysin now under study, listeriolysin O, is a member of a family of thiol-activated cytolysins present in 15 diverse species of Gram-positive bacteria which in addition to *Listeria* include members of the genera *Bacillus*, *Clostridium* and *Streptococcus*¹⁵. Many of these bacteria are pathogenic including *S. pyogenes*, *S. pneumoniae*, *C. perfringens* and *C. tetani*. The study presented here raises the intriguing possibility that other bacteria may at some stage in their life-cycle adopt an intracytoplasmic niche. □

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Independent transfer of mitochondrial chromosomes and plasmids during unstable vegetative fusion in *Neurospora*

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THE sporadic distribution of similar introns in organelle, nuclear ribosomal RNA and bacteriophage genes suggests that at least some of these introns are mobile genetic elements¹⁻³. Some plasmids in fungal mitochondria contain intron-like sequences⁴ and, like introns, they have scattered distributions within and among species⁵⁻⁷. The occurrence and evolutionary importance of such horizontal transfer of DNA, not only between fungi, but among a wide range of organisms have been matters of much discussion^{1-3,8-12}. Here, we report experimental evidence for transfer of *Neurospora* mitochondrial plasmids from one mitochondrial genotype to another at high frequency during unstable vegetative cell fusion. Exchange of mitochondrial and nuclear genomes can also occur. These observations suggest that vegetative fusion may have a more important role in the mitochondrial genetic structure of natural populations than is generally thought, and may provide an explanation for the distribution of certain plasmids and possibly other mobile genetic elements.

The Mauriceville-1c (refs 4, 13) and Varkud-1c (ref. 14) natural isolates of *Neurospora* contain closely related versions of a mitochondrial plasmid (referred to here as the V plasmid) which shows some similarities to organelle introns and retroelements^{4,14,15}. We have shown recently that Varkud-1c also contains a second, smaller plasmid (designated VS) which is unrelated in nucleotide sequence to the V plasmid, except that it also contains sequences similar to the core of Group I introns²⁶.

We have examined the distribution of the V and VS plasmids in natural isolates of *Neurospora* (Fig. 1). Including Mauriceville-1c (FGSC# 2225) and Varkud-1c (FGSC# 1823), the V plasmid has been found in five *N. intermedia* isolates from India (1805, 1809, 1821, 1823, 2653); one *N. sitophila* isolate from India (2492; data not shown); and two *N. crassa* isolates, one from Pakistan (1825) and one from Texas, USA (2225). Similar cross-species distributions have been described for other *Neurospora* plasmids⁶. Six of these isolates (1805, 1809, 1821, 1823, 2653, shown in Fig. 1c, and 2492, not shown) also contain the VS plasmid. Thus, although the V plasmid can occur without the VS plasmid, we have never observed the VS plasmid in an isolate without the V plasmid. This suggests that the VS plasmid may depend upon the V plasmid for some aspect of its replication or maintenance.

It is of interest to determine how what is essentially the same, rather rare, V plasmid could be distributed in only a few isolates that are so geographically distant (India, the USA), and genetically divergent (representing three different species and showing substantial differences in the restriction enzyme digestion patterns of their mitochondrial chromosomes). We decided to investigate the possibility that the V and VS plasmids could transfer from one mitochondrial chromosomal genotype to another during vegetative cell fusion (heterokaryon formation). Complementary nuclear auxotrophic genetic markers (panthothenate or nicotinamide requirement) were introduced into an isolate of *N. intermedia* that contained the plasmids (1805) and one that lacked plasmids (2495). The mitochondrial chromosomes of these strains are distinguishable by a 1 kilobase (kb) restriction fragment length polymorphism (RFLP) which, by Southern hybridization, seems to be due to the presence or absence of an optional intron in the gene encoding apocyto-

chrome *b* (C. A. Reynolds and R.A.C., unpublished data). The short RFLP is present in 2495 and is designated cobS; 1805 contains the long form, cobL (Fig. 2). The absence of the V and VS plasmids and the presence of the cobS RFLP in the strains that were to serve as plasmid recipients was confirmed by Southern hybridization (Fig. 2).

Heterokaryon formation was attempted, using standard procedures¹⁶, between pairwise combinations of a [cobS]nic-1 and [cobL V VS]pan-2 strain on agar-solidified minimal medium (see Fig. 3 legend). Either of the auxotrophic 'parents' alone grew by a few millimetres at most and stopped, whereas all of the heterokaryons formed a mycelium and conidiated in 3-10 days. Several observations indicated that the heterokaryons were extremely unstable, as might be expected because *Neurospora* has a multilocus, vegetative incompatibility system that effectively prevents formation of stable heterokaryons between strains whose alleles differ at any of these loci^{17,18}. Some heterokaryons failed to grow when subcultured onto fresh minimal medium; those that did, grew more slowly and conidiated more poorly than is usually observed with compatible heterokaryons. In heterokaryons in which one parent contained a nuclear albino mutation in addition to the auxotrophic marker, growth of albino sectors indicative of very unbalanced nuclear ratios was common. Consistent with the instability of the heterokaryons, we have never obtained heterokaryotic single-conidiospore isolates from these cultures, indicating that most and possibly all of the mycelium was sectored for one or the other nuclear type.

To obtain stable, viable cultures for further analysis, single conidial derivatives, now homokaryotic for either the pan-2 or the nic-1 nuclear type, were isolated from nine heterokaryons (Fig. 3). With respect to the genotypes of the nucleus and mitochondrial chromosome, both of the parental types ([cobL]pan-2: 19C, 21L, 23K, 36N, 37K, 63L, 63M and 80M; and [cobS]nic-1: 19A, 21A, 22B, 32C, 36D and 37E) and one of the non-parental combinations ([cobL]nic-1: 23D, 63G, 63H, 80H) were observed among the single conidial isolates analysed (Fig. 3a). Failure to observe the other non-parental combination may be due simply to the small number of isolates analysed or it may reflect the incompatibility of this nucleus/mitochondrion combination. Nonetheless, the recovery of any non-parental combinations suggests that even though the heterokaryons were very unstable, the parental strains must have remained fused at least long enough for mixing of mitochondrial and nuclear populations to occur. If such fusions can occur in natural populations, this might provide a mechanism for exchange of mitochondrial genomes even between individuals whose nuclear genotypes may be very different.

Mitochondrial DNAs from each of these single conidial derivatives were analysed by restriction digestion and Southern hybridization with cloned V and VS plasmid DNAs to determine whether the plasmids had been gained by any of the mitochondria that had previously lacked them (Fig. 3b, c). Of the six informative isolates (i.e., those that contained [cobS] mitochondrial DNA characteristic of the plasmid-minus parent), five had gained the V plasmid (19A, 22B, 32C, 36D and 37E; Fig. 3b). The VS plasmid was present in four of these (19A, 22B, 32C and 36D; Fig. 3c) and absent from 21A and 37E. These observations indicate that efficient transfer of the plasmids from one mitochondrial type to the other is occurring even in very unstable fusions. This mode of plasmid transfer could account for the occurrence of very similar plasmids in otherwise distantly related *Neurospora* isolates⁵⁻⁷.

In one conidial isolate from heterokaryon 37 (37E in Fig. 3), the V plasmid transferred without the VS plasmid. The V plasmid was also found to be present and the VS plasmid absent in ten additional [cobS]nic-1 single conidial isolates from this heterokaryon (data not shown). If the V plasmid can transfer into another mitochondrial chromosomal genotype independently of the VS plasmid under natural conditions, isolates such

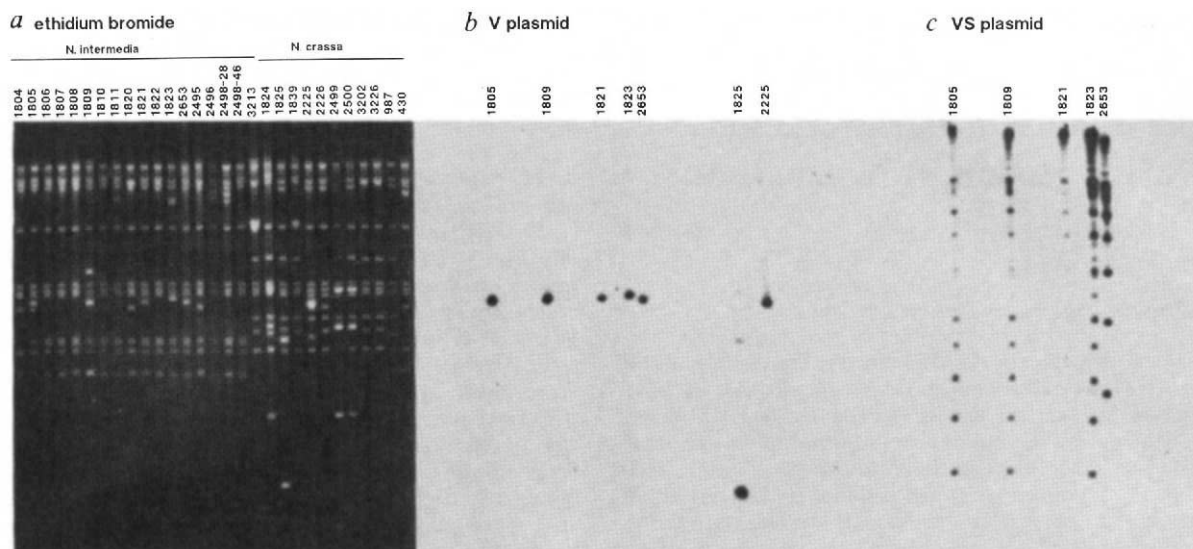
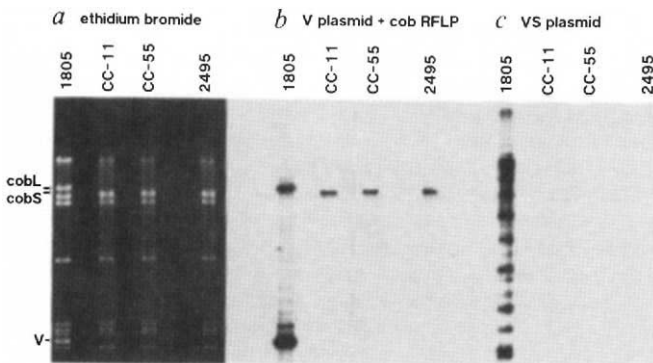


FIG. 1 Distribution of the V plasmid among natural isolates of *Neurospora*. **a**, Ethidium bromide-stained agarose gel of mitochondrial DNAs digested with *EcoRI*. Isolates were obtained from the Fungal Genetics Stock Center. *N. intermedia* isolates (with their FGSC numbers in parentheses) are: Golur-1b (1804); Golur-1c (1805); Kadakola-1 (1806); Kadakola-1g (1807); Kurubara Shettihally-1f (1808); Kurubara Shettihally-1 (1809); Kalastwadi-1c (1810); Kalastwadi-1 (1811); Mysore-1 (1820); Mysore-1e (1821); Varkud-1b (1822); Varkud-1c (1823); Nandi Hill 1-4a (2653); Chickkadana-1 (2495); Chickkadana-1j (2496); and two single conidial isolates of Pulikeezhu-1 (2498-28 and 2498-46) which differ from each other by the presence or absence of a Group II intron in the mitochondrial *col* gene (A. Sommerfeld and R.A.C., unpublished); Fred-6 (3213). All except Fred-6, which is from Texas, USA, were collected from India. *N. crassa* isolates are: from Pakistan, Lahore-1 (1824); Lahore-1b (1825); from India, Aarey-1e (2499); Aarey-1g (2500); from Texas, USA, Mauriceville-1c (2225); Mauriceville-1d (2226); Spurger-7 (3203); Saratoga-11 (3226); from Africa, Adiopodoume (formerly North Africa I; 430); laboratory strain 74A (987). **b**, **c**, Autoradiographs of Southern

transfers²⁰ of the gel in **a** which have been hybridized with cloned plasmid probes. **b**, Transfer hybridized with pV4, which contains a complete monomer-length V plasmid from Varkud-1c (as well as a small fragment that hybridizes to the *cob* region of the mitochondrial chromosome, which accounts for the slight hybridization to *EcoRI*-3; see Fig. 2). *EcoRI* cleaves the Varkud-1c allele of the V plasmid into a 2.7 kb fragment (and six small fragments, which have migrated off the gel shown here). **c**, Transfer hybridized with pD7, a clone which contains a complete monomer-length VS plasmid. *EcoRI* does not cleave the VS plasmid²⁶; the hybridization pattern shows the multimeric series of bands typical of *Neurospora* mitochondrial plasmids¹³. **METHODS**. Mycelia were grown in liquid Vogel's medium according to standard procedures¹⁶. DNA was isolated from flotation-gradient-purified mitochondria²¹ by the UNSET-phenol method²². DNAs were digested, electrophoresed in the presence of ethidium bromide to confirm the extent of digestion and sample loading, transferred to nitrocellulose membrane, and hybridized with ³²P-labelled probes according to standard procedures²³. Detection was by autoradiography on Kodak X-Omat AR-5 film.

FIG. 2 Construction and characterization of strains for heterokaryon formation. *EcoRI*-digested mitochondrial DNAs from the plasmid-containing isolate 1805, the plasmid-minus isolate 2495 and the two of its progeny (CC-11 and CC-55), which were used for heterokaryon formation (Fig. 3), were electrophoresed on a 0.7% agarose gel. The ethidium bromide staining pattern is shown in **a**. DNAs were then transferred to nitrocellulose and hybridized with probe pV4 (**b**) which contains the complete V plasmid and a small fragment of the mitochondrial chromosome that detects the *cob* RFLP; or probe pD7 (**c**) which contains the VS plasmid.

METHODS. Natural isolate 2495 was crossed as the female parent with *N. crassa* laboratory strain RCK-11 *nic-1 nic-1 al-2*, which is auxotrophic for nicotinamide and is albino. As the mitochondrial chromosome is inherited exclusively from the maternal parent in *Neurospora*^{12,24,25}, each of the individual progeny from this cross (designated CC-1, CC-2, etc.) contains the 2495 mitochondrial chromosome ([*cobS*]; confirmed by restriction enzyme analysis in Fig. 2a). Progeny containing the nuclear *nic-1* mutation were identified for use in heterokaryon formation; some progeny also contained the recessive nuclear albino mutation *al-2*: this allows unstable heterokaryons to be recognized visually by the presence of albino and orange (wild-type colour) sectors in the mycelium. A cross with 1805 as the female parent and strain 45 *A nic-1 pan-2 al-2* (auxotrophic for pantothenate and nicotinamide) as the male was performed to construct strains (designated AA-1, AA-2, etc.) that contain the 1805 mitochondrial chromosome ([*cobL*]), the V and VS mitochondrial plasmids, and the nuclear *pan-2* mutation. The mitochondrial genotype is enclosed in square brackets; such strains will be designated as [*cobS*]*nic-1* and [*cobL* V VS]*pan-2*. As is typical of inter-specific crosses, both ascospore formation and germination were poor (about two orders of magnitude less than with intra-specific crosses), but sufficient to allow recovery of viable progeny. See Fig. 1 legend for additional methods.



as Mauriceville-1c which contains the V but not VS plasmid could have acquired the V plasmid from a donor that contained both plasmids.

Transfer of the linear *kalilo* mitochondrial plasmid between *Neurospora* strains during vegetative growth using a slightly different procedure (growth on sorbose-containing media) has also been demonstrated (B. Cheng, D. A. Court and H. Bertrand, personal communication). Transfer of a plasmid between mitochondria during protoplast fusion in *Brassica* has also been observed¹⁹.

The recent study by May and Taylor¹² of transfer of a different *Neurospora* mitochondrial plasmid showed that this plasmid can be transmitted independently of the mitochondrial chromosome at low, but evolutionarily significant, frequencies by the male parent during sexual crosses; our work shows that independent transfer can also occur during unstable vegetative fusion. However, in neither case has it been shown that transfer actually occurs through sexual contact or vegetative fusion, respectively. Indeed, we wonder if the low frequency of transfer observed during crossing could also be the result of infrequent, unstable, fusion between the female and male parents, which are present together for several days during the cross.

Vegetative fusion is generally considered to be unimportant to the genetic structure of natural *Neurospora* populations because the large number of incompatibility loci and high level of allelic polymorphism at these loci would prevent formation of stable heterokaryons^{17,18}. Our results show that stable

heterokaryons are not a prerequisite for major changes in the mitochondrial genetic system. Transfer of the V and VS plasmids and exchange of nuclear and mitochondrial genomes occurs so efficiently under laboratory conditions that even extremely rare and unstable fusions may contribute to the horizontal transfer of mitochondrial DNA, plasmids and possibly other mobile elements in nature. □

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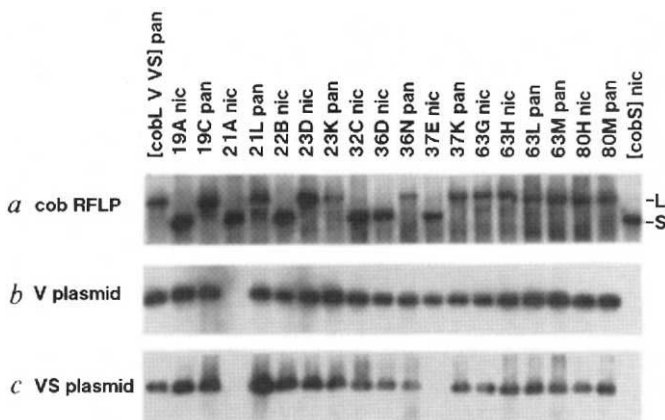


FIG. 3 Analysis of mitochondrial chromosomes and plasmids in single conidiospore isolates derived from heterokaryons. Mitochondrial DNAs were prepared from single conidial isolates (in most cases, one *nic-1* and one *pan-2* isolate) from each of nine heterokaryons and analysed by Southern hybridization. The extreme left and right lanes contain mitochondrial DNA from strains used to form the heterokaryons: [cobL V VS]*pan-2* and [cobS]*nic-1*, respectively. *a*, *Pst*I digests hybridized with a cloned restriction fragment of the mitochondrial *cob* gene (pNB1905); *b*, *Eco*RI digests hybridized with pV4 which contains the complete V plasmid and a fragment of the *cob* gene; *c*, *Hae*III digests (which linearize the VS plasmid) hybridized with the cloned VS plasmid (pD7).

METHODS. Heterokaryon formation was attempted by the standard procedure¹⁶ of co-inoculating conidia from pairwise combinations of either CC-11 ([cobS]*nic-1 al-2*) or CC-55 ([cobS]*nic-1*) and one of several AA strains ([cobL V VS]*pan-2*) onto 1 ml slants of agar-solidified minimal medium in 10 × 170 mm test tubes. A small amount of conidia (and hyphal fragments) was then subcultured to a fresh minimal slant. Nine heterokaryons, each designated simply by a number (19, 21, 22, 23, 32, 36, 37, 63 and 80) were analysed. Conidia from this subculture of each heterokaryon were suspended in water and plated on sorbose-containing media supplemented with nicotinamide, pantothenate or nothing (minimal) and allowed to form colonies. Nicotinamide-requiring and pantothenate-requiring single conidial isolates (now homokaryotic) were recovered from each heterokaryon, and identification of auxotrophic markers was confirmed by testing for growth on each of these three media. Cultures of each single conidial isolate are designated by a capital letter following the number of the heterokaryon from which they were derived (for example, 19A, 19C, etc.). See Fig. 1 legend for additional methods.

Hyperthermophilic archaeobacteria within the crater and open-sea plume of erupting Macdonald Seamount

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HYPERTHERMOPHILIC archaeobacteria, found in terrestrial and submarine hydrothermal areas^{1–6}, thrive at temperatures between 80 and 110 °C and are unable to grow below 60 °C. They represent life at the known upper temperature limit. On the continent, their biotopes are solfataric fields and hot springs. Within the marine environment, hyperthermophilic archaeobacteria have been found in shallow-water hydrothermal fields as well as in deep hot sediments and vents^{1,2,4–6}. They include strictly anaerobic sulphidogenic and methanogenic chemolithoautotrophs as well as fermentative and sulphidogenic heterotrophs, and are important as primary producers and consumers of organic matter within high-temperature ecosystems. Their distribution and possible modes of dissemination are at present unknown. Here, we report for the first time on a community of hyperthermophilic archaeobacteria within the active zone of an erupting submarine volcano⁷, and on its spread through the ensuing cooled-down open-ocean

TABLE 1 Samples taken at Macdonald Seamount

Date (1989)	Designation of sample	Station/dive	Distance from active zone (km)	Depth (m)	pH	CH ₄ (nl l ⁻¹)	Description of sample
24 January	MD 1	Cy 28 MS	2	37	8.24	41.5	2 litres sea water
24 January	MD 2	Cy 28 MS	2	68	8.30	62.2	4 litres sea water
24 January	MD 3, 4	—	4	0	8.02	ND	5 litres sea water
26 January	MD 7	—	0.5	0	8.10	ND	Metallic grey slick floating in patches at the surface (100 ml; about 50% water)
27 January	MD 8	Cy 35 MS	1.0	130	6.07	7,828	1.5 litres sea water from the plume, strong odour of H ₂ S
27 January	MD 9	Cy 36 MS	0.3	136	6.23	7,828	5 litres sea water from the plume, strong odour of H ₂ S
27 January	MD 10	Dive No. 1013	0.01	120	ND	ND	Lava rocks with orange-red hydrothermal precipitates collected from the active crater wall

Resazurin (1 mg l⁻¹) was added to samples MD 1, MD 2, MD 3, 4, MD 8 and MD 9 as redox indicator. To protect possible anaerobic hyperthermophiles, samples were reduced by addition of sodium dithionite¹³. Microbial cells were concentrated by passage through nitrocellulose ultrafilters (0.1 µm pore width). Filters were then placed into sterile 28-ml serum tubes which were filled with reduced sea water from the same sample. The tubes were tightly sealed and stored at 4 °C. The slick sample (MD 7) was poured into a 100-ml storage bottle. After reduction¹³, the bottle was stoppered and stored at 4 °C. The rocks of sample MD 10 were crushed on board the R/V *Le Suroit* and were transferred into a sterile 100-ml storage bottle which was then filled with sterile reduced sea water. The bottle was tightly stoppered and stored at 4 °C. At the end of the cruise (5 February 1989), all samples were carried to the home laboratory by air without temperature control. ND, not determined.

plume. Most of the organisms are close relatives of species previously known only from a submarine solfataric field in Italy, whereas others are new. By their conversion of volcanic gases and sulphur at high temperatures, archaeobacterial communities living in seamounts may be important participants in marine ecological, geochemical and volcanic processes.

During the 1989 French-German expedition to Macdonald Seamount (*Cyana-Le Suroit*, J.L.C. *et al.*, unpublished) we wished to explore potential high-temperature microbial ecosystems. Macdonald Seamount is a submarine 'hot spot' volcano in Polynesia (28°58.7'S, 140°15.5'W, refs 8, 9 and J.L.C. *et al.*, unpublished), approximately 40 m below the sea surface. Just as we approached the Macdonald area, the volcano began to erupt⁷. We therefore were able to explore for the first time an erupting submarine volcano and its surroundings for the presence of hyperthermophilic archaeobacteria.

On the first day of eruption we took hydrocast samples about 2 to 4 km away from the active site (MD 1; MD 2; MD 3, 4; Table 1). pH was between 8.30 and 8.02 and methane concentra-

tion varied between 41.5 and 62.2 nl l⁻¹, which are normal values for surface open sea water¹⁰.

After one quiescent day eruptions began again, and large patches of greyish slick of metallic appearance floated on the surface (J.L.C. *et al.*, unpublished). Some of this material was taken as a sample (MD 7; Table 1). Next day there were further strong eruptions, interrupted by periods of silence⁷. Hydrocast samples were taken from water showing green discoloration at the surface (MD 8 and MD 9; Table 1). They had abnormally low pH values of around 6 and extremely high methane concentrations of up to 7,800 nl l⁻¹, indicating that they came from the submarine plume of the eruption. On the same day during a quieter period, sampling within the active crater was attempted by the submersible *Cyana* (dive No. 1013). Some lava rocks with orange-red precipitates from the crater wall were collected (MD 10; Table 1), but sampling ended when the volcano erupted again (ref. 7 and J.L.C. *et al.*, unpublished).

To test the samples of water and rock for hyperthermophilic bacteria, anaerobic sterile culture media were inoculated with the samples (2.5% inoculation) which were then incubated at 75 °C and 92 °C in the presence of an H₂/CO₂ (80:20; 300 kPa) and N₂/CO₂ (80:20; 300 kPa) atmosphere. Culture media consisted of artificial sea water supplemented with acetate, peptone and yeast extract with and without elemental sulphur¹¹⁻¹³. Surprisingly, culture media inoculated with samples MD 7, MD 8, MD 9 and MD 10 became turbid after only 15 h incubation, indicating an unexpected high initial concentration of hyperthermophilic bacteria. Microscopic inspection of the enrichment cultures revealed high concentrations of mixtures of coccoid cells with heterogeneous morphology. The mixtures consisted of motile and non-motile irregular cocci, wedges, and plates, about 0.3–5 µm in width. Under the ultraviolet microscope, at 420 nm, some of the cells showed a blue-green fluorescence characteristic of methanogens and archaeobacterial sulphate reducers^{4,12}. During growth, all cultures formed H₂S (between 0.5 and 6 µmol ml⁻¹). After transfer into fresh medium, some organisms within enrichment cultures MD 7, MD 8 and MD 10 were also able to grow at 100 °C and 102 °C. However, none of the enriched bacteria grew at 60 °C or below, indicating that they consisted exclusively of hyperthermophiles¹⁴. No hyperthermophiles were enriched from seawater samples MD 1, MD 2 and MD 3, 4. This shows the absence, or extremely low occurrence, of such organisms in open-ocean water (fewer than 10 cells l⁻¹).

To estimate the concentration of hyperthermophiles in sample MD 7, enrichment medium was inoculated by multiple dilutions

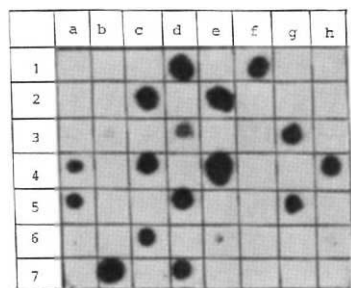


FIG. 1 Dot-blot DNA/DNA hybridization of enrichment cultures with *Thermococcus celer*. In analogy to Grunstein's bacterial colony hybridization (ref. 15; A. Neuner and K.O.S., unpublished), cell masses (3×10^9) of enrichment cultures and pure cultures of marine hyperthermophilic archaeobacteria were dotted onto a hybridization membrane (colony/plaque screen, NEN). The DNAs were liberated, denatured, and fixed to the membrane according to Grunstein and Hogness¹⁵. Hybridization with *in-vitro* labelled [³²P] DNA from *Thermococcus celer* (2.65×10^6 c.p.m. per µg DNA)¹⁵ was under 'optimal' conditions: 24 h at 65 °C in buffer $3 \times$ SSC with 24% formamide²³. Hybridization signals were recorded by autoradiography (24 h, Konika A2 X-ray film). Dot-blots of the following enrichments and pure cultures are shown: MD 7 (3b, 4h, 6c, 7b); MD 8 (1f, 3d, 3g, 5a, 6e); MD 9 (2c, 4a, 4c, 5d, 6h); MD 10 (2e, 5g, 7d); *Thermococcus celer* (1d, 4e); *Staphylothermus marinus* (5f); *Archaeoglobus fulgidus* (6a); *Pyrococcus furiosus* (7e); and *Pyrodicticum occultum* (7f).

TABLE 2 Identification and concentrations of cells of hyperthermophilic members of archaeobacterial genera present in samples taken during Macdonald Seamount eruption

Sample*	Members of genus (viable cells l ⁻¹):			
	<i>Pyrodicticum</i>	<i>Pyrococcus</i>	<i>Thermococcus</i>	<i>Archaeoglobus</i>
MD 7	10 ⁴	10 ⁶	10 ⁶	10 ⁴
MD 8	+	+	+	+
MD 9	0	0	+	0
MD 10	0	+	+	+

For enrichment of hyperthermophilic archaeobacteria, the anaerobic culture technique of Balch and Wolfe¹² was used. Stoppered 120-ml serum bottles, containing 20 ml of different anaerobic cultivation media¹¹⁻¹³ were inoculated with 0.5 ml of the samples. The cultures were incubated without shaking at 75 °C or 92 °C. The cell concentrations of members of hyperthermophilic archaeobacteria within sample MD 7 were determined by enrichment cultures obtained from serial dilutions. Members of different genera of hyperthermophilic archaeobacteria were identified by dot-blot DNA/DNA hybridization as described in Fig. 1. DNA probes of the following strains were used: *Thermococcus celer* (DSM 2476; ref. 20), *Pyrococcus furiosus* (DSM 3638; ref. 21), *Pyrodicticum occultum* (DSM 2709; refs 1, 13), *Archaeoglobus fulgidus* (DSM 4304; refs 4, 22), and *Staphylothermus marinus* (DSM 3639; ref. 18). +, Qualitatively determined; 0, not present.

* See Table 1.

of MD 7 and incubated at 75 °C. The 1:1000 dilution still gave rise to a positive enrichment culture (1 ml inoculation). Therefore, the volcanic slick floating in the open sea contained the very high concentration of at least 10⁶ hyperthermophilic archaeobacteria per litre. The other samples seemed inhomogeneous (containing rock pieces or filters from processing the samples; Table 1) and determination of cell concentrations seemed valueless. In view of the rapid development of all enrichment cultures, the initial cell concentrations (inocula) must have been of the same order of magnitude.

To identify possible relatives, enrichment cultures were probed against pure cultures of known marine hyperthermophiles by dot-blot DNA/DNA hybridization (Fig. 1, Table 2, refs

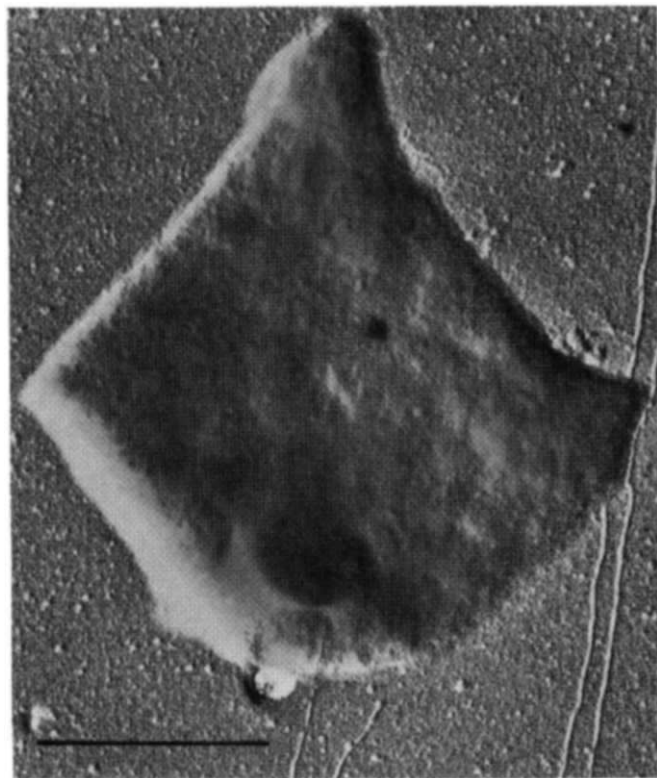


FIG. 2 Electron micrograph of a platinum-shadowed cell of a novel hyperthermophile isolated from slick sample MD 7. The culture was grown anaerobically at 100 °C for 24 h in sea water containing yeast extract and elemental sulphur in the presence of an H₂/CO₂ atmosphere (80:20; 300 kPa)¹³. Scale bar 1 µm.

15-17). To our surprise, very strong signals became visible with the DNA of *Pyrodicticum occultum*, *Pyrococcus furiosus*, *Thermococcus celer* (Fig. 1) and *Archaeoglobus fulgidus* (Table 2). These organisms have only been found so far within a hydrothermal system off Vulcano, Italy. The strong hybridization indicates the presence of close relatives of these species within samples MD 7, MD 8, MD 9, and MD 10 (Table 2). In contrast, no relatives of *Staphylothermus marinus* were detected (data not shown)¹⁸.

Within the slick sample MD 7, cell concentrations of members of the genera *Pyrodicticum*, *Pyrococcus*, *Thermococcus* and *Archaeoglobus* were determined to be about 10⁴, 10⁶, 10⁶ and 10⁴ cells per litre, respectively (Table 2). In addition to relatives of already known hyperthermophilic bacteria, Macdonald Seamount contains organisms which are unique. From sample MD 7 at 100 °C, a highly irregular organism was isolated (Fig. 2) which does not show DNA homology with any of the probes (data not shown).

Our investigation presents the first evidence for a high-temperature microbial ecosystem at an erupting submarine volcano. The Macdonald community of hyperthermophilic archaeobacteria contains anaerobic H₂-oxidizing members of *Pyrodicticum* and *Archaeoglobus* which gain energy by sulphur and sulphate respiration respectively. As these organisms are able to use CO₂ as the sole carbon source, they are producers of organic material within the hot volcanic environment. Hydrogen, CO₂, sulphur and sulphate serve as essential nutrients and are present within the volcanic gases and seawater¹⁹. The Macdonald community of hyperthermophiles also consists of anaerobic, strictly heterotrophic members of the genera *Pyrococcus* and *Thermococcus* and of the novel isolate MD 7, which can grow by fermentation or sulphur-respiration on cell homogenates, proteins, sugars and amino acids and are, therefore, consumers of organic matter. Because it was impossible to take any more samples in the erupting crater, the microbial analyses are still incomplete and the ecosystem must be further explored.

Detection of high concentrations of hyperthermophilic archaeobacteria within the cooled-down submarine plume and floating volcanic slick about 1 km away from the active zone shows the release of these organisms during submarine volcanic eruptions and a possible mode of transport. The unexpected finding that the Macdonald hyperthermophiles are close relatives of the Italian organisms may be explained by spreading and long-term survival of hyperthermophiles at relatively low temperatures during dissemination. This is in agreement with our findings that members of the genera *Pyrodicticum*, *Pyrococcus*, *Thermococcus* and *Archaeoglobus* can be enriched from samples even after storage for years at 4 °C in the presence of oxygen (K.O.S., unpublished). □

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Probing the calcium-induced conformational transition of troponin C with site-directed mutants

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THE contraction of skeletal muscle is regulated by calcium binding to troponin C (TnC; Fig. 1)¹. TnC consists of two spatially independent domains, each of which contains two metal ion binding sites^{2–5}. Calcium binding to the regulatory sites of the N-terminal domain^{6,7} triggers muscle contraction by a series of conformational changes. Site-directed mutagenesis offers a means of elucidating the links in this signal path between TnC and actin–myosin crossbridges. Such mapping is possible if the mutants shift the equilibrium between 'on' and 'off' states of the regulatory complex while maintaining the coupling between calcium binding and tension development. Candidate amino-acid residues for yielding this information would be in positions remote from the calcium-binding sites and from the site of development of tension. Analysis of the crystal structure of TnC^{2,8,9} and of the model of the calcium-activated molecule¹⁰ has enabled us to identify two such residues: Glu 57 and Glu 88. In separate experiments we have replaced each of these residues by lysines. The resulting reduction in calcium affinity indicates that these residues have a long-range effect on calcium binding. This result may reflect the formation of a salt bridge between positions 57 and 88 that is not present in the native

molecule. Moreover, the level of tension recovery when the mutants are incorporated into muscle suggests that the interaction between TnC and other muscle components has also been altered. Thus, these residues may participate in the contraction signal transmission.

A model for the calcium-induced conformational change has been proposed¹⁰, based on the crystal structure of TnC from turkey skeletal muscle^{2,8}. In the crystal, the regulatory sites (I and II) are devoid of metal ions, whereas the structural sites (III and IV) are occupied by calcium ions. The model proposes that on calcium binding, the N-terminal regulatory domain assumes a similar conformation to that of the C-terminal domain, such that the interhelical angle opens in each of the two EF hand motifs. As a result of this conformational change, the intramolecular carboxyl–carboxylate interaction between Glu 57 (helix C) and Glu 88 (helix D) of the second EF-hand motif is no longer possible (Fig. 1). This interaction is energetically favourable only at a pH lower than 6 (the crystals are obtained at pH 5). Further evidence for the change in the relative position of these residues upon calcium binding comes from studies of calcium affinity at pH 5.3 showing that only the high affinity sites bind calcium at this pH (ref. 11). We have carried out mutagenesis experiments aimed at displacing the calcium binding equilibrium towards the apo-state at neutral pH by replacing Glu 57 with a lysine residue. If the model is correct, a salt-bridge should then be formed between positions 57 and 88. The alternative salt-bridge-forming mutant (Glu 88 to Lys) was also constructed. These mutants are thus expected to produce a reduction in calcium affinity of the regulatory sites at physiological pH, hence necessitating an increase in calcium concentration for the activation of skinned fibres reconstituted with these mutant TnCs.

Large amounts of wild-type and mutant chicken TnC were expressed in *Escherichia coli* and purified to homogeneity¹² (Fig. 2). Calcium binding was measured directly using ⁴⁵Ca in an equilibrium dialysis assay⁶. When compared with the wild-type TnC, the E57K mutant showed identical binding curves at high pCa values, corresponding to sites III and IV, and a displacement towards higher calcium concentrations for the low-affinity sites I and II (Fig. 3a). Thus, the changes in calcium affinity of E57K are specific and restricted to the N-terminal domain. Moreover, all four sites are still present in the mutant proteins, as saturation occurs at approximately 4 moles of calcium per mol of TnC. The mutant protein E88K shows a larger shift towards higher calcium concentrations at low pCa when compared with E57K (Fig. 3b). A small shift was also observed at high pCa values. The significance of this secondary effect is

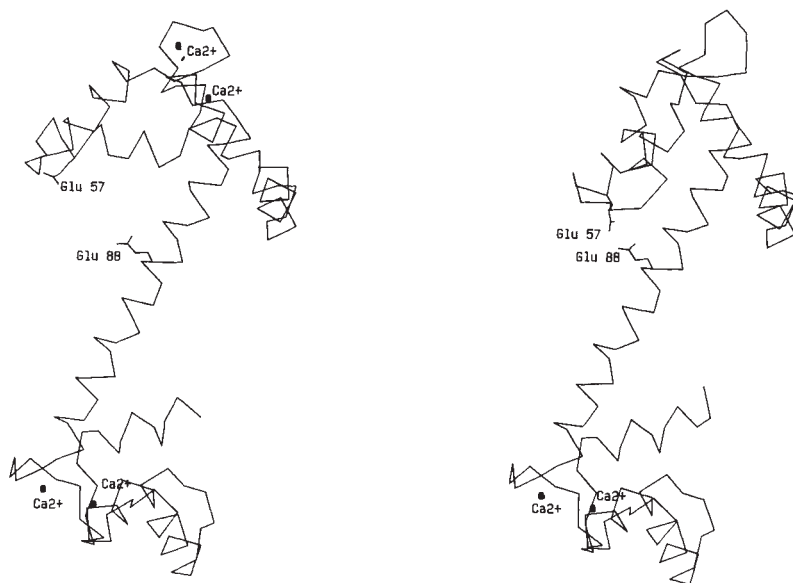


FIG. 1 Alpha-carbon representation of the crystal structure of TnC (right) and of the calcium activated model (left). The side chains of Glu 57 and Glu 88 are shown, as well as the positions of the calcium ions. The separation of the N- and C-terminal domains by an inter-domain α -helix is apparent. The second EF motif of TnC is made of helix C, Ca^{2+} -binding loop II and helix D. Glu 57 is located on helix C and Glu 88 on helix D. This interaction is stable at the pH of the crystal (5.0). Replacing it by a salt bridge should stabilize the apo state at neutral pH.

unknown, but could be because position E88 is located in the long D/E helix linking the two domains. Thus, as predicted, these mutants, which lie outside the calcium binding loops and about 20 Å from the closest Ca^{2+} position, specifically reduce calcium affinity. The fact that both substitutions have a similar effect on calcium binding strengthens the hypothesis that their interaction with each other results in the formation of a salt bridge.

Analysis of the regulatory behaviour of the mutant TnCs in the native contractile apparatus was done by replacing the endogenous TnC of permeabilized muscle fibres with each of the mutant proteins. Segments of single muscle fibres derived from chicken pectoralis or rabbit psoas muscle were mounted on a tension transducer and isometric tension was recorded as a function of pCa. To ensure maximum replacement by the recombinant proteins, endogenous TnC was extracted until calcium-activated tension was reduced to 10% or less of the original value, and the fibre was reconstituted with one of the recombinant TnCs. After a second pCa-tension curve was collected, the incorporated TnC was extracted and replaced with the other recombinant TnC in order to collect the final set of tension data.

Consistent with other reports^{13,14}, after exhaustive extraction of endogenous TnC, reconstitution either with the wild-type recombinant TnC or with the protein purified from chicken skeletal muscle restores only part (up to 70%) of the original tension and is accompanied by decreased calcium sensitivity. This change in sensitivity may reflect incomplete occupancy of TnC binding sites on the thin filament, as shown previously^{15,16}. Therefore, it is important to compare calcium sensitivity conferred by mutant TnCs with that conferred by wild-type recombinant TnC: a pCa-tension curve that superposes on the curve of the recombinant wild-type TnC means that the mutation has no detectable effect. This is the case for a mutant in which Glu 132, in the C-domain, was replaced by Lys (Fig. 4a).

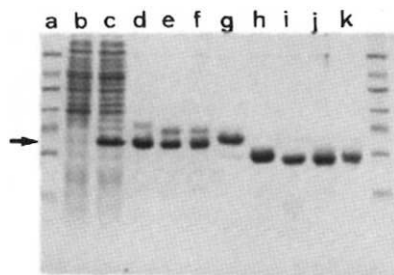


FIG. 2 Coomassie blue-stained SDS-polyacrylamide (15%) gel of the purified proteins used in this study. *a*, Molecular mass markers of 66,000 (66K), 45K, 36K, 29K, 24K, 20K and 14.2K. *b*, Total cell extract of *E. coli* containing the plasmid pLICITnTn before the heat shock. *c*, Total cell extract of *E. coli* after induction by heat shock. Purified fusion TnC before cleavage with factor Xa: *d*, wild type; *e*, E57K; *f*, E88K; *g*, E132K. The band above each of the first three purified fusion proteins is the magnesium-free form¹². Purified TnC after cleavage with factor Xa and purification: *h*, wild type; *i*, E57K; *j*, E88K; *k*, E132K.

METHODS. The construction of the expression vector, the preparation of TnC mutants and their expression has been described¹². We have modified the purification and digestion protocols to exclude detergents from the preparation. After induction and growth for 12–16 h, the bacteria from each litre of culture were collected, resuspended in 30 ml buffer (50 mM Tris-Cl pH 8.0, 25% sucrose, 1 mM EDTA) and lysed by three passes through a 'French press' at 16,000 p.s.i. The lysate was cleared at 27,000g for 30 min, the volume of supernatant was increased to 50 ml l⁻¹ culture and TCA was added to give a final concentration of 5%. The purification was continued as described¹². In the chromatographic purification, the urea concentration was increased from 6 to 8M and the salt gradients were changed to 60–250 mM KCl in the first DE-52 column and to 0–300 mM KCl in the DE-52 column used to remove the N-terminal peptide. Digestion with factor Xa was carried out¹² in 25mM Tris-Cl pH 8.0, 100 mM NaCl, 2 mM MgCl₂ and 4 mM CaCl₂.

Calcium-activated tension development by skinned muscle fibres reconstituted with E57K and E88K shows that these mutants can restore calcium regulation of tension, demonstrating that both mutant proteins can interact with the other components of the regulatory system (Fig. 4). The mutant proteins decrease the calcium sensitivity of the preparation, however. This effect is highly reproducible: it is observed with both mutants, in fibres from rabbit or chicken muscle, and irrespective

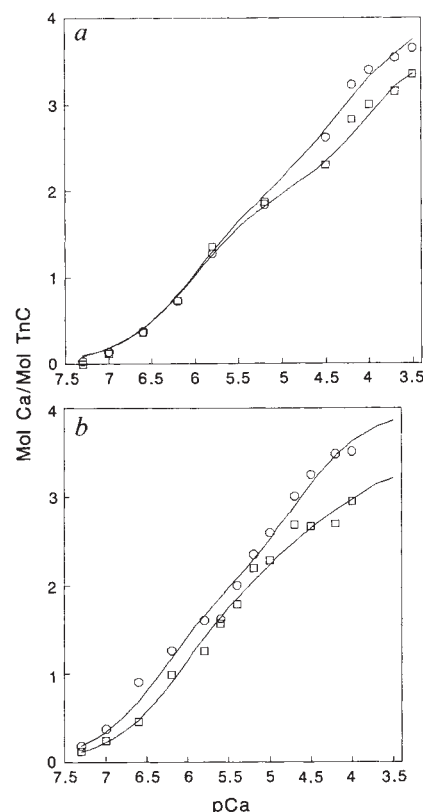


FIG. 3 Calcium binding to wild-type (○) and mutant (□) E57K (*a*) and E88K (*b*). Calcium binding to each mutant was determined in parallel with the corresponding data for wild-type TnC. The experimental data points were fitted (continuous lines) using the procedure described⁶. The binding constants obtained are (in M⁻¹): *a*, wild type ($K_1=K_2=1.1 \times 10^6$, $K_3=K_4=2.1 \times 10^4$), E57K ($K_1=K_2=1.0 \times 10^6$, $K_3=K_4=8.1 \times 10^3$). *b*, Wild type ($K_1=K_2=2.2 \times 10^6$, $K_3=K_4=4.5 \times 10^4$), E88K ($K_1=K_2=1.2 \times 10^6$, $K_3=6.2 \times 10^4$, $K_4=1.5 \times 10^3$).

METHODS. Calcium binding to mutant and wild type TnCs were determined in parallel by equilibrium dialysis using pure TnC (*a*) or fusion TnC (*b*)^{6,17}. A solution with 2 mg ml⁻¹ TnC was pre-dialysed for 48 h against three changes of 10 mM imidazole, 100 mM KCl, 0.1 mM EGTA and 2 mM MgCl₂, pH 7.0, at 4 °C. For each protein, samples of 0.5 ml were distributed into 1.5-ml plastic tubes, each covered with a dialysis membrane. Pairs of tubes containing the wild type and mutant proteins were equilibrated for 60 h at 25 °C against each pCa solution. Tracer calcium concentration was kept constant at 0.04 μCi ml⁻¹. The amount of cold CaCl₂ used to obtain the desired free calcium concentration was calculated using the computer program SPECS (ref. 18). The absolute constants for the binding of EGTA species to Ca^{2+} (in log values, $K_1=10.869$; $K_2=5.33$), Mg^{2+} ($K_1=5.21$; $K_2=3.37$) and H^+ ($K_1=9.526$; $K_2=8.928$; $K_3=2.8$ and $K_4=2.12$) at 25 °C were converted to apparent constants using the program ABSTOAPP¹⁸. The contamination of calcium in the water was determined to be 10⁻⁶ M by atomic absorption spectroscopy. The stocks of KCl, MgCl₂ and CaCl₂ were titrated with Hg(NO₃)₂ in the presence of diphenylcarbazone to determine their actual concentrations. Corrections were made for the calcium added as ⁴⁵Ca and for the increase in volume due to the addition of calcium. Duplicate samples of 150 μl from the inside and outside of the dialysis tube were counted. The protein concentration in each tube was measured after dialysis and corrected for the degree of contamination. Calculations were performed as described^{6,17}.

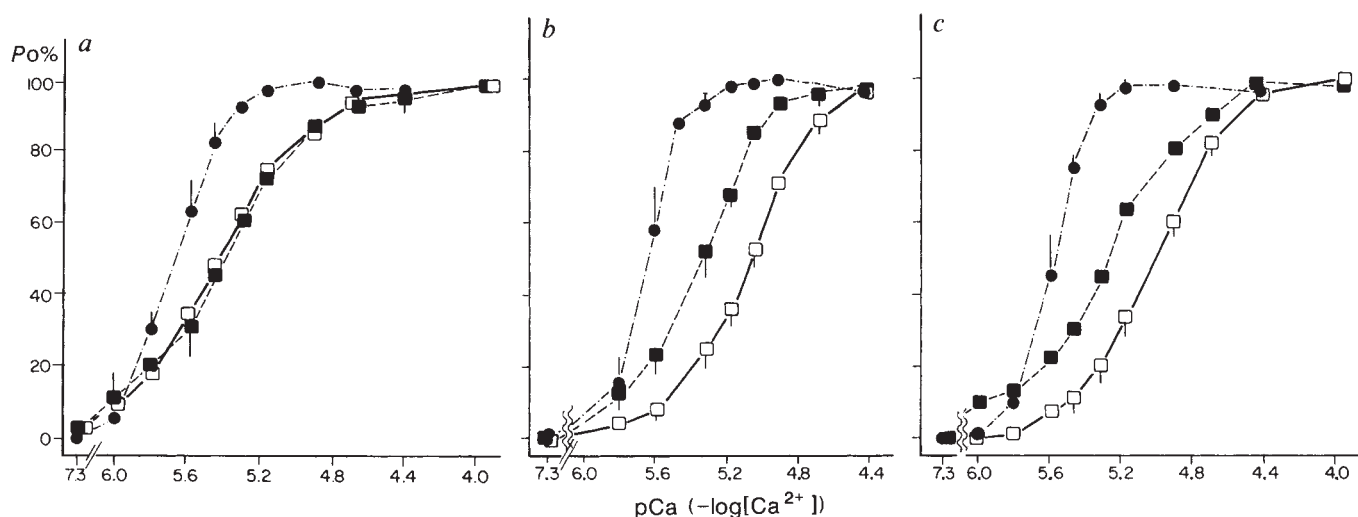


FIG. 4 Normalized tension/calcium concentration curves obtained from isolated rabbit skinned muscle fibres before and after reconstitution with recombinant TnC. Isometric tensions were measured before TnC extraction (●) and after reconstitution with wild type (■) and mutant TnC (□). Each curve is normalized to its own maximum. Calcium concentration is indicated as $-\log [\text{Ca}^{2+}]$ (pCa). *a*, Skinned fibres reconstituted with wild type ($n=2$) and mutant E132K ($n=3$) (this mutant was used as a control as it has the same charge modification as E57K and E88K but in a region which does not affect calcium binding). *b*, Skinned fibres reconstituted with wild-type and mutant E57K ($n=4$). *c*, Skinned fibres reconstituted with wild-type and mutant E88K ($n=5$). Bars, s.e.m.

METHODS. After the pCa-tension curve with endogenous TnC, chemically skinned fibres^{19,20} looped between an isometric tension transducer²⁰ and a movable hook were washed every 10 min at 30 °C with an EDTA solution of low ionic strength¹⁵, until maximum Ca-activated tension (P_0 , at pCa 4.4) was reduced to 10% of the original value. Rapid stirring was maintained throughout the experiment. Fibres were reconstituted for 30 min at 20 °C in 0.6–0.7 ml of a relaxing solution containing 0.2–0.4 mg ml⁻¹ mutant or wild-type TnC, 0.1 mM dithiothreitol, 20 mM Tris-Cl, 140 mM K-propionate, 5 mM K₂EGTA, 10 mM imidazole propionate, 6.1 mM Mg acetate and 4.4 mM K₂Na₂ATP at pH 7.0 (calculated MgATP²⁻, 4 mM; free Mg²⁺, 2 mM)²¹. After a second pCa-tension curve, the fibre was re-extracted to a tension less than 10% P_0 and reconstituted with the second TnC protein, and a third pCa-tension curve was obtained. pCa-tension curves were obtained

at 20 °C in a medium similar to that used for reconstitution, but without dithiothreitol and Tris and with part of the EGTA replaced by CaEGTA²¹. After reconstitution all pCa-tension curves were shifted to the right and had reduced slopes. The decrease in slope has been attributed to decreased interaction between successive regulatory units along the thin filament¹⁵. In our experiments neither higher protein concentrations nor longer reconstitution times restored the original slope or calcium sensitivity even when TnC purified from chicken muscle was used to reconstitute the fibres. We therefore compared the fibres reconstituted using wild-type recombinant TnC with fibres reconstituted using mutant TnC; usually wild type and one mutant were tested in each fibre. The pCa-tension curve obtained with the original TnC (before extraction) is included for comparison. The same results were obtained using chicken skinned fibres reconstituted with E57K and E88K. Sensitivity to the activating cation also decreased when Sr²⁺ replaced Ca²⁺ as the activating cation. With Sr²⁺ the half-maximum tension shifted from pSr 4.4 (fibres reconstituted with recombinant wild-type TnC) to pSr 3.8 (fibres reconstituted with mutants E57K or E88K). As a control for the effects of partial tension recovery, 17 fibres were partially extracted until their maximum tension was decreased to the levels observed with the reconstituted fibres. These fibres had a shift in the mid point of the pCa curve of 1.7 ± 0.2 -fold when compared with native fibres. Fibres reconstituted with E88K with comparable levels of tension had a shift of 3.4 ± 0.6 -fold when compared with native fibres.

of the order in which the proteins are reconstituted into the fibre. The shift corresponds to a 1.5- to 2-fold increase in calcium concentration necessary to obtain half of the maximum tension, which is compatible with the direct measurements of calcium binding shown in Fig. 3. Fibres reconstituted with the mutants develop similar maximum tensions with strontium as with calcium, but are four times less sensitive to strontium than fibres containing recombinant wild type TnC (data not shown).

The level of incorporation of both E57K and E88K mutants is lower than that of the recombinant wild-type TnC, as detected by maximum tension obtained in each case. Of the two mutants, E57K consistently provides a greater tension recovery (80% of wild type) than E88K (48%). Yet the decrease in calcium sensitivity is identical for the two mutants. The partial recovery of tension observed in E57K and E88K cannot be responsible for the shift in calcium sensitivity as the mutant E132K also shows partial tension recovery but no shift in calcium sensitivity (Fig. 4). We propose that Glu 88 is more closely involved in contacts with other proteins of the muscle system, such as troponin I. It is not surprising that such a significant change of charge alters the association between the proteins, and therefore the level of reconstitution.

Our experiments demonstrate two aspects of TnC function: first, charge-charge interactions remote from the calcium binding sites can be used to influence calcium affinity. Second, the initial step in the chain of events linking calcium binding to

muscle contraction is a conformational change of the N-terminal domain of TnC, involving an opening of the angles between the EF-hand helices. The experiments also implicate Glu 88 in the association of TnC with the other members of the troponin-tropomyosin complex, possibly troponin I. □

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New ventures in freeze drying

L. Rey

Called lyophilization because it produces dry products that 'love' solvents (lyos-philein), this technique consists of a successive set of operations, each of which is critical.

FREEZE drying, also called lyophilization, is a technique used for the stabilization of delicate aqueous substances, essentially biologicals, which cannot be stored at room temperature. It is a multistage operation during which a product is frozen under controlled conditions, then dried at low temperature by direct sublimation of ice, followed by progressive desorption of 'bound' water.

Product preparation

Substances to be processed have, first, to be prepared in solutions at a given concentration in order to produce a consistent, solid plug after drying. For high-activity, diluted biologicals such as hormones, enzymes and vaccines, it is necessary to add 'bulking' agents such as lactose, mannitol, glycine, dextran, polyvinylpyrrolidone or methylcellulose to give added structural strength.

Freezing process

The material is then hardened by low-temperature freezing. In this step, water crystallizes out into ice while the solutes and 'active' substances become progressively embedded into a complex network of eutectics, eutectoids, amorphous and rigid glass-like structures. In the course of this evolution, they support serious stresses, such as hypertonic shocks from concentrating salts, mechanical pressure from ice crystals, segregation from differential crystallization and pH changes.

Living cells and delicate biologicals are sensitive to these changes and need to be protected by appropriate additives to prevent alterations of membranes, protein tertiary structure, or other fine morphological or biochemical properties that play an essential role in biological activity and potency.

The choice of protective additive is difficult and has to be made in conjunction with a given descending temperature profile. Macromolecules help reduce crystal size and associated constraints, sugars bind some water — essential for the maintenance of biological properties — into unfreezable forms, and monosodium glutamate and dimethyl sulphoxide protect micro-organisms. Extensive research has been done in this field and has been reviewed by Mazur¹. It emphasizes the specific requirements of freeze drying, which prevent the use of high-quality protectors, such as glycerol, because of their inability to dry.

In all cases, it is essential to achieve a state of utter rigidity in order to provide long-term stability and to allow for successful drying from the frozen state². This necessitates the use of not only very low temperatures, but also the application of complex freezing procedures with intermediate rewarming steps — so called 'thermal treatments'³ — in order to rupture metastable equilibria. This is necessary because the final structure is more dependent upon the 'thermal history' of the sample than on its temperature alone.

Drying process

When the material has reached the maximum temperature of complete solidification, it can be dried from the frozen state. This operation is carried out in two successive steps, usually under vacuum.

Step 1: sublimation

At first, energy is cautiously fed into the system to provide the necessary heat for direct sublimation of the ice. The ice-crystal network disappears progressively as the sublimation interface sinks into the frozen core, leaving behind the porous 'ghost' of the initial structure. By this 'carving' action, performed under vacuum, a vast internal surface is developed into the product (often tens of square meters per gram of dry substance), giving it its 'lyophile' character but also conditioning its exquisite sensitivity to gas contamination. During this period, the product temperature should be kept in the frozen mass under the minimum temperature of incipient melting to prevent interstitial softening⁴. The energy feed has, thus, to be kept under constant control, which implies adjustment of both heat flow and vacuum level, as well as permanent monitoring of the physical status of the processed substance by methods such as the measurement of its electric impedance in a.c. voltage.

After completion of the primary drying process, about 90 per cent of the initial water content has been lost. It, then, is necessary to extract most of the remaining unfreezable 'bound' water to secure total stabilization of the product for long-term preservation.

Step 2: Isothermal desorption

In this second step, the last 10 per cent or so of remaining water is progressively desorbed under high vacuum until the

appropriate residual moisture content is reached. This is a long and tedious operation, as it is difficult to drive energy into a porous matrix placed under vacuum that behaves like an almost perfect insulating material. Overheating and even scorching should be avoided, and this is why, in practical terms, the extraction of this residual water takes almost as long as the initial sublimation phase. The optimal final moisture content varies according to the substance and its later use. In general, complex proteins can be dried to a 1–2 per cent residual moisture without impairment, although the long-term preservation of biological standards may require levels as low as 0.1–0.5 per cent. Living cells should not be overdried and have to be adjusted to a 2–3 per cent level. Close monitoring and control of this phase is important and can be achieved by intermittent equilibrium water-vapour measurements or by thermal conductivity.

Final conditioning

When desorption is completed, the dry product, held under vacuum, is ready to undergo final conditioning. The key, then, is to prevent contamination by oxygen and water vapour and to ensure that the product is kept hermetically sealed. To that end, it has been the practice to seal the vials or ampoules containing the product directly under vacuum. Today, however, the preferred method is to place it under a controlled atmosphere of dry, neutral gas, such as nitrogen or argon. This is also an important step because of the high activity of the vast, bare internal surface carved by sublimation and further cleaned by desorption. The first contact with a gas is critical as it will be stamped immediately on the product and will condition its long-term evolution.

The above procedures are further complicated when handling injectable therapeutic agents. In such cases, freeze drying has to be carried out under stringent sterile conditions in order to avoid contamination by pyrogens, micro-organisms or particulate material. This has led to the development of instruments and systems with a one-way, no-return flux of products and *ad hoc* in-place cleaning and sterilization facilities.

Product usage

If prepared as above, freeze-dried products can be stored at room temperature,

preferably without light, for long periods, while still retaining most, if not all, of their essential original properties. Vaccines, sera, enzymes, hormones, monoclonal antibodies, international biological standards, antibiotics, tissue extracts, steroids, micro-organisms and viruses for therapeutic, diagnostic or scientific use can be processed and stabilized by lyophilization. They are easy to store, ship and handle, although for certain delicate vaccines, it is advisable to carry out the procedure under controlled low temperatures, even in sub-zero conditions⁵.

Freeze-dried products can be reconstituted to their original state at any time by the addition of water. They can also be redissolved in balanced salt solutions or any other physiological fluid at the desired concentration.

Another possibility is to use freeze drying as a first, non-disturbing step within a complex extraction process. For example, water can be eliminated by lyophilization from highly hydrated marine organisms, and the resulting freeze-dried material extracted directly by organic solvents.

New technologies

Because of its unique characteristics, freeze drying offers many new opportunities, and some of the more innovative examples follow.

Selective filters

Because of the high internal surface that develops with lyophilization, it can be resorted to prepare an expanded tri-dimensional matrix, which can be used later on as an almost absolute filter to retain sub-micronic particles, or as a selective plug that can absorb given chemicals when inserted within a liquid or gas flow stream⁶.

New galenic tool

For the same reason, freeze drying can be used to prepare otherwise stable chemicals in the form of porous tablets of ultra-fast dissociation. This gives rise to pure or mixed solutions or suspensions of finely dispersed active substances with high biodisponibility and combined flash and prolonged effects (Lyoc)⁷.

Non-aqueous media

The extension, in 1964, of lyophilization to non-aqueous media⁸ opened up an entirely new field of investigation. Because of the use of organic solvents such as dioxane, chloroform, diethylamine, benzene and carbon tetrachloride, different products such as phospholipids, fats and organic polymers can be dried by sublimation from the frozen state.

It has also been shown that it is possible to use mineral solvents, such as carbon dioxide and ammonia, which demonstrate interesting properties for the transfer of

Microbiological materials

Next week's American Society for Microbiology Annual Meeting in Anaheim, California, promises a broad selection of products to meet the needs of microbiologists, including a benchtop bioreactor and instrumentation for measuring O₂ uptake and CO₂ production.

THE American Type Culture Collection is offering complete catalogues of bacteria, phages and algae, and protozoa as an **on-line database service** (Reader Service No. 101). The ATCC on-line service can be accessed through the CODATA Microbial Strain Data Network and includes information on strain descriptions, instructions on maintenance of cultures, media formulations and special uses for specific strains. Other services accessible with the ATCC on-line database include ATCC recombinant DNA materials collection and the ATCC/NIH repository of human and mouse DNA probes and libraries. Electronic mail, international microbial and hybridoma databases and electronic conferences are additional features available to users of the on-line service.

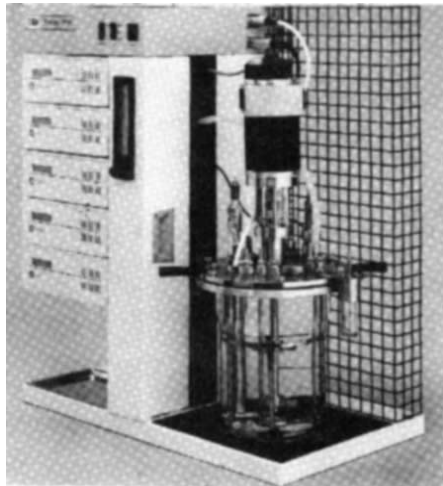
Whittaker Bioproducts is offering a choice of media formulations with its new line of powdered media to suit all microbial applications (Reader Service No. 102). Milled in an inert environment for maximum stability and minimum degradation due to chemical oxidation, says Whittaker, the powdered media is tested for endotoxins, cell-growth promotion, pH, residual moisture and osmolality.

So-Low Environmental Equipment, manufacturers of **ultra-low temperature freezers**, has introduced the Model A18-

120 freezer with an 18 cubic foot capacity designed for preserving tissue, plasma, blood cells and microbial samples (Reader Service No. 103). The 35 × 47 × 75-inch upright freezer provides adjustable temperature control from -18 to -85 °C. Other features include an air-cooled condenser, a temperature recorder and a buzzer-alarm system.

Optimum environments

To meet the needs of cell culture specialists, LH Fermentation has introduced the Series 210 modular and microprocessor-controlled **benchtop bioreactor** (Reader



LH Fermentation's benchtop bioreactor.

aromas (CO₂) and the preparation and preservation of free radicals (NH₂)⁹.

Special microstructures

The combined use of selected solvents can aid in the development of highly regular structures of very small grain size and low density. One example of this, is the coating of glass microspheres, used as targets in laser-induced thermonuclear fusion, by a thin, homogeneous shell of porous dextran freeze-dried from a solution of 45 per cent dioxane in water, which does not change volume during freezing¹⁰.

The same technology also allows the preparation of complex structures by the simultaneous or successive freeze drying of different solutes in separate solvents, each one leaving an internal network of thin septa, regularly intermingled within a tri-dimensional matrix. These supports could be of great interest for the selective retention of organic chemicals, or as

supports for mineral or biological catalysts¹¹.

This clearly demonstrates that freeze drying is still evolutive and likely to offer many new opportunities in basic and applied sciences. □

Louis Rey, Scientific Advisor, Ancien Elève de l'Ecole Normale Supérieure, Agrégé de l'Université, Rue Marteney 74, CH 1005 Lausanne, Switzerland. For more information, fill in reader service number 100.

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Service No. 104). Optimized for microbial and animal cell applications, the open design of the bioreactor can accommodate up to seven control and pump modules for temperature, dissolved oxygen, anti-foam, pH/redox and agitation speed. Modules for mass flow, weight and carbon dioxide will be available shortly. The bioreactor will accept autoclavable glass vessels with capacities from two to ten litres. For monitoring variables during the process, the bioreactor can be connected to an IBM AT or PS/2 running Bio-pc software. This configuration can manage up to four bioreactors simultaneously and log up to eight measured variables, with a minimum logging interval of one minute. The agitator drive, which operates at 10–250 r.p.m., is designed to provide optimum operating conditions for cell growth.

Optimize the growth of microbial cultures, says New Brunswick Scientific, with its Innova 3000 microprocessor-controlled **waterbath shaker** (Reader Service No. 105). Shaker speeds can be set to between 25 and 400 r.p.m. and controlled to within 1 r.p.m., says NBS. The Innova 3000 is powered by a brushless d.c.

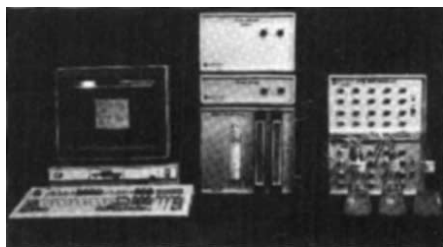


There's a whole lot of shaking going on with the Innova 3000 from NBS.

motor and features a counter-balanced triple-eccentric gyrotory drive. The temperature can be controlled to within $\pm 0.1^\circ\text{C}$ over the $5\text{--}40^\circ\text{C}$ range and to within $\pm 0.5^\circ\text{C}$ over the $40\text{--}99.9^\circ\text{C}$ range. User-defined setpoints are stored in a non-volatile memory. Additional features of the Innova 3000 shaker include: self-diagnostic status lights, safety alarms and an adjustable water-level control for the automatic replenishment of evaporated water.

Dealing with data

Measure O_2 consumption and CO_2 production simultaneously in ten chambers with Micro-Oxymax, Columbus Instruments' **metabolic computer** (Reader Service No. 106). Offering a sensitivity of $0.2\ \mu\text{l h}^{-1}$, Micro-Oxymax provides improved accuracy over the conventional Warburg apparatus, says Columbus, and can accommodate measuring chambers with capacities ranging from 100 ml to 10



Columbus Instruments' Micro-Oxymax measures O_2 consumption/ CO_2 production.

litres. The measuring intervals are user-selectable and can range from 5 minutes to 24 hours. Micro-Oxymax runs under full IBM PC control. Data for the volume of O_2 consumed and CO_2 produced, together with the measuring chamber's temperature, can be output as hard copy or stored to disk. Some of the stated applications include measurement of insect metabolism, detection of pest infestation in grain, proliferation of bacteria or moulds, and the O_2 uptake of tissue cultures and fermenters.

The manual recording of results in microbial assays could be a thing of the past with Dynatech's latest piece of imaging hardware — the Omnicon **zones of inhibition analyser** (Reader Service No. 107). In the time that it takes to record the potency of one antibiotic, the Omnicon computerized image analysis system and specially designed software can automatically enter the results from 12 antibiotics into a database, says Dynatech. Omnicon



Eliminate manual data recording of microbial assays with Omnicon.

achieves fast throughput: three-, four- and five-point assays can be performed in just five minutes using the menu-driven system.

To meet differing reporting and data handling requirements using a wide range of assay techniques and a variety of instruments, Wellcome Diagnostics has introduced a **software package** called Wellconet (Reader Service No. 108). Wellconet software can accept data from various methods of data input: manually,

bar-code readers and laboratory instruments. The software interfaces with a wide range of sample processors and plate readers. Comprehensive data analysis is provided by Wellconet, says Wellcome, with data output as hard copy or transmitted to another computer. Wellconet is



Software solutions to data handling.

designed to support up to 70 microplate-based assays, including qualitative, quantitative, kinetic and haemagglutination tests.

In a spin

In need of a centrifuge but lacking in benchspace? Jouan has introduced three new microprocessor-controlled, compact **floor-model centrifuges** in non-refrigerated, refrigerated and thermostatted forms (Reader Service No. 109). Occupying less than four square feet, all three models can reach up to $4,200\text{ g}$ with a horizontal rotor and up to $9,000\text{ g}$ with a fixed-angle rotor. By simply changing the plastic inserts, the centrifuges can accommodate tubes as small as 0.5 ml , or bottles as large as 750 ml . Special carriers are available for 250-ml conical bottles and microtitre plates, and aerosol-containment buckets provide a safe means of handling pathogens and toxic samples. The GR422 refrigerated model provides temperature control from -8°C to $+40^\circ\text{C}$, whereas the GT422 thermostatted model combines a heating and refrigeration system, providing chamber temperatures from -8°C to $+50^\circ\text{C}$. Microprocessor control allows the user to program parameters for speed or RCF, time, temperature, temperature deviation, acceleration and braking rates. Programs can be stored in one of ten memories.

International Biotechnologies has brought out a new line of **microcentrifuge** with built-in safety features that protect the user from contamination by aerosols and liquid spills (Reader Service No. 110). Available with variable- and constant-speed operation, the IMV-13 and IMV-15 operate at variable speeds of up to 13,500 and 15,000 r.p.m., respectively. The IMC-15 provides a constant speed of

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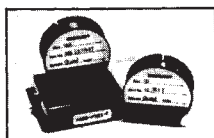
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15,000 r.p.m. All three models reach the maximum speed within five seconds, and have a quick-spin feature for short spins to provide rapid access to samples. All three can be equipped with rotors that accommodate 16, 18 or 24 sample tubes. The



IBI's centrifuge with safety in mind.

IMV-13, however, has extra capacity and can hold up to 48 sample tubes. The microcentrifuges are fitted with a disposable polyethylene chamber liner that can be removed in the event of accidental spills. As an additional safety feature, most of the rotors can be fitted with optional aerosol containment covers.

Media filtering

For media filtering applications, Nalge says its Nalgene 90-mm **bottle-top filters** provide a greater throughput than traditional 50-mm membranes (*Reader Service No. 111*). The filter's cellulose acetate membrane is available in two pore sizes: 0.2 µm and 0.45 µm. Characteristically, the membranes exhibit low protein binding, high flow rates and little or no loss of specific proteins, says Nalge. The filters attach directly to media bottles with 33- to 45-mm necks by deep screw threads, providing a secure fit that is suitable for vacuum filtration. Available in 500-ml

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sizes, the non-cytotoxic filters are radiation-sterilized and ready for use.

Zapcap **bottle-top filters** represent the latest addition to Schleicher and Schuell's line of filtration products (*Reader Service No. 112*). The membrane and support assembly is designed to provide flow rates that are five times faster than that of conventional 500-ml bottle-top filters. Zapcap filters are suitable for the sterilization or clarification of aqueous solutions such as media, serum or cell-culture supplements. The one-piece disposable unit fits glass media bottles with 33- to 45-mm necks. Each non-pyrogenic, non-cytotoxic filter unit contains a low protein binding cellulose acetate membrane. Also included, is a pre-filter, which can be placed on top of the membrane to maximize the throughput of proteinaceous solutions.

Odds and ends

Gadget-minded people may be interested in Tecnomara's answer to the Bunsen burner for flaming loops — the Rondo-flame **automatic loop sterilizer** (*Reader Service No. 113*). Rondo-flame holds the inoculation loop above and at an angle to the flame, ensuring complete sterilization even in the area around the securing nut, says Tecnomara. As an alternative to the Bunsen, the company states that Rondo-flame can reduce gas consumption by up to 80 per cent, producing standardised results every time.

Branson Ultrasonics is offering a new **ultrasonic cell disruptor** — the Sonifier II — designed for ultrasonic disintegration and homogenization (*Reader Service No. 114*). The ergonomically designed Sonifier II has a built-in broadband tuning circuit, which eliminates the need for manual retuning after changing probes or for critical applications. The Sonifier II is available in two power levels: the Model 250 (200 W) and the 450 (400 W), which is recommended for heavy-duty use. For processing heat-volatile solutions and temperature-sensitive material, the instrument's pulser mode can be used, which applies ultrasonic energy to a solution at a rate of one pulse per second: pulse duration can be set to between 0.1 and 0.9 seconds. Stated applications include disintegrating bacteria and yeast, disrupting cells and tissues, isolating subcellular organelles, preparing liposomes and releasing viruses from tissues. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.